X ● C07K 14/18 W 93AC ANO

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/51, 15/62, A61K 39/29
C12Q 1/61, C07K 13/00, 15/28
G01N 33/576

A2

(11) International Publication Number: WO 93/10239

(43) International Publication Date: 27 May 1993 (27.05.93)

(21) International Application Number: PCT/GB92/02143

(22) International Filing Date: 20 November 1992 (20.11.92)

(30) Priority data:
9124696.7 21 November 1991 (21.11.91) GB
9213362.8 24 June 1992 (24.06.92) GB

(71) Applicant *(for all designated States except US)*: COMMON SERVICES AGENCY [GB/GB]; Trinity Park House, South Trinity Road, Edinburgh EH5 3SE (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : SIMMONDS, Peter [GB/GB]; 1 Glengyle Terrace, Edinburgh EH3 9LL (GB). CHAN, Shui-Wan [GB/GB]; 10 Kilmaurs Road, Edinburgh (GB). YAP, Peng, Lee [GB/GB]; 5 Meadow Place, Edinburgh EH9 1JZ (GB).

(74) Agents: McCALLUM, William, Potter et al.; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).

(81) Designated States: AU, CA, FI, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

Published
Without international search report and to be republished upon receipt of that report.

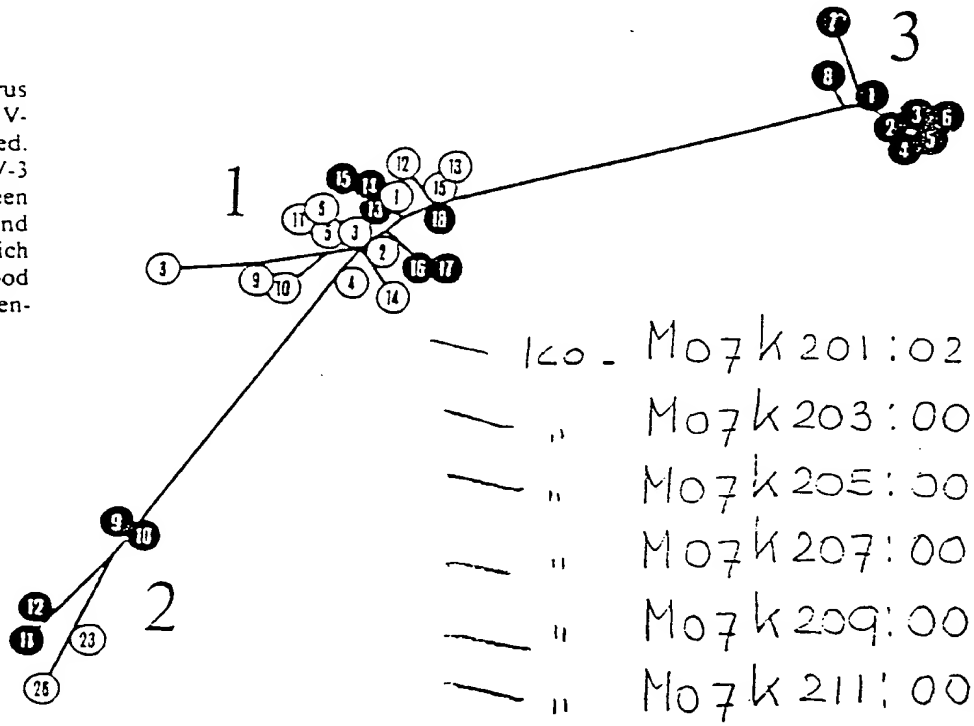
C12Q 1/70 B6A

XCC

(54) Title: HEPATITIS-C VIRUS TESTING

(57) Abstract

New styles of hepatitis-C virus (HCV), referred to as HCV-3 and HCV-4, have been identified and sequenced. Antigenic regions of HCV-2, HCV-3 and HCV-4 polypeptides have been identified. Immunoassays for HCV and antibodies thereto are described, which allow more complete screening of blood samples for HCV, and allow HCV genotyping.



- 1C0 - M07K201:02
- " M07K203:00
- " M07K205:00
- " M07K207:00
- " M07K209:00
- " M07K211:00

HEPATITIS-C VIRUS TESTING

Technical Field

The present invention relates to the discovery of new types of hepatitis C virus, that we have termed type 3 (HCV-3) and type 4 (HCV-4). In particular, it relates to the etiologic agent of hepatitis C virus type 3 and 4, and to polynucleotides and immunoreactive polypeptides which are useful in immunoassays for the detection of HCV-3 and HCV-4 in biological samples; and also to the use of antigenic HCV-3 and HCV-4 specific polypeptides in vaccines.

Background of the Invention

Acute viral hepatitis is a disease which may result in chronic liver damage. It is clinically diagnosed by a well-defined set of patient symptoms, including jaundice, hepatic tenderness, and an increase in the serum levels of alanine aminotransferase and aspartate aminotransferase. Serologic immunoassays are generally performed to diagnose the specific type of viral causative agent.
Historically, patients presenting with symptoms of hepatitis and not otherwise infected by hepatitis A, hepatitis B, Epstein-Barr or cytomegalovirus were clinically diagnosed as having non-A, non-B hepatitis (NANBH) by default.

clone was ligated with overlapping clones to produce a larger viral antigen (C100) corresponding to part of the NS3-NS4 genomic region. C100 was then fused with the human superoxide dismutase (SOD) gene, expressed in use as a large recombinant fusion protein (C100-3) and used on solid phase to develop radio-labelled (RIA) and enzyme-linked immunosorbent assays (ELISA).

Polynucleotides useful for screening for HCV are disclosed in European Patent Specification EP-A-0398748. European Patent Specification EP-A-0414475 purports to disclose the propagation of HCV in culture cells and the production of antigens for use in diagnostics. European Patent Specification EP-A-0445423 discloses an improved immunoassay for detecting HCV antibodies.

Blood banks in the United Kingdom have recently begun routine testing of blood donors for antibodies to components of HCV. One assay involves the detection of HCV antibodies to C100-3 polypeptides. The C100-3 antibody recognises a composite polyprotein antigen within non-structural regions of the virus and is a consistent marker of HCV infection. However, in acute infections this antibody is unreliable because of the delay (typically 22 weeks) in seroconversion after exposure. Furthermore, the C100-3 antibody test lacks specificity for the hepatitis C virus.

Second generation antibody tests employ recombinant antigens or synthetic linear peptides representing

analysis. The invention has thus identified polynucleotide sequences and polypeptides which are HCV-3 and HCV-4 specific. These may be used to diagnose HCV-3 and HCV-4 infection and should thus be included in any definitive test for HCV infection.

One aspect of the invention provides polynucleotide sequences unique to hepatitis C virus types 3 and 4 (HCV-3 and HCV-4). The sequences may be RNA or DNA sequences. In principal any HCV-3 or HCV-4 specific polynucleotide sequence from non-coding, core, E1, E2 or NS1-5 genome regions can be used as a hybridisation probe. The sequences may be recombinant (i.e. expressed in transformed cells) or synthetic and may be comprised within longer sequences if necessary. Equally, deletions, insertions or substitutions may also be tolerated if the polynucleotide may still function as a specific probe. Polynucleotide sequences such as core, NS3, NS4 and NS5 which code for antigenic proteins are particularly useful.

Another aspect provides an antigenic HCV-3 or HCV-4 specific polypeptide, particularly from the core, NS3, NS4 or NS5 regions (e.g. the HCV-3 or HCV-4 counterparts of C100 polypeptide, 5-1-1 polypeptide, C33 polypeptide or C22 polypeptide or epitopes thereof) or polypeptides including these antigens.

A further aspect of the invention provides labelled antigenic HCV-3 or HCV-4 specific polypeptide (or mixtures thereof, particularly from the core and NS4 regions) for use in an immunoassay.

In particular, the invention allows blood donor screening by conventional assays (using HCV type 1 encoded antigens) to be supplemented with a second test that contains two oligopeptides corresponding to first and second antigenic regions found in the NS-4 sequence of HCV type 3 (positions 1691 to 1708; sequence KPALVPDKEVLYQQYDEM and positions 1710 to 1728; sequence ECSQAAPYIEQAQVIAHQF) and two derived from the equivalent regions of HCV type 2, R(A/V)V(V/I)(A/T)PDKE(I/V)LYEAFDEM and ECAS(K/R)AALIEEGQR(M/I)AEML.

The corresponding HCV-4 antigens from substantially positions 1691 to 1708 and 1710 to 1728 may be used for HCV-4 detection.

Thus, the present invention has also identified corresponding polynucleotide and polypeptide sequences which may be used to identify hepatitis C type 2 viral infection.

Production and detection of the antigen-antibody immune complex may be carried out by any methods currently known in the art. For example, a labelling system such as enzyme, radioisotope, fluorescent, luminescent or chemiluminescent labels may be employed, usually attached to the antigen. Labelled anti-antibody systems may also be used. The recombinant antigen may be either used in liquid phase or absorbed onto a solid substrate.

Oligopeptides corresponding to the antigenic regions of all three major types may also be used separately to serologically distinguish individuals infected with

non-coding region of HCV samples from 18 blood donors and a comparison with previously published nucleotide sequences (see Table 2); sequence numbering corresponding to the prototype HCV-1 sequence (ref.4) and previous designations of type 1 or 2 being indicated;

Figure 2 is a phylogenetic analysis showing clustering of the sequences into three types viz; HCV-1, HCV-2 and HCV-3 for the 5' NCR results of Figure 1 using the maximum likelihood algorithm, shown as an unrooted tree. Numbers 1-18 in full circles correspond to blood donor sequences E-b1 through E-b18. Numbers 1 to 26 in open circles correspond to the previously published sequences identified in Table 2.;

Figure 3 is a comparison of deduced amino acid sequences in the NS-5 region of blood donors (E-b1, E-b2, E-b3, E-b7 (type 3) and E-b12 (type 2) with those previously published (Table 2). Amino acid residue numbering follows that of the HCV-1 polyprotein (4) and uses single letter amino acid codes;

Figure 4 is a phylogenetic analysis of the NS-5 region using the maximum likelihood algorithm, shown as an unrooted tree. Symbols are as described for Figure 2;

Figure 5 is a comparison of deduced amino acid sequences in the NS-3 region of blood donors (E-b1, E-b2, E-b6, E-b7 (type 3) with those previously published (Table 2). Group 1/1: amino acid sequence of f1, f3, f4, f5, h2, h3, h4 (one), i2, i3, i4, p1, p2; Group 1/2:

cysteine; Q: glutamine; E: glutamic acid; G: glycine; H: histidine; I: isoleucine; L: leucine; K: lysine; M: methionine; F: phenylalanine; P: proline; S: serine; T: threonine; W: trytophan; Y: tyrosine; V: valine."-": sequence not determined; difference from consensus shown in bold.

Figure 10(a) shows a comparison of amino acid sequences between residues 1679 and 1768 (Choo et al., 1991) of the three major variants of HCV. T16, T42, T77, T1801, T1825: Scottish blood donors infected with HCV type 1; T351: Scottish blood donor infected with HCV type 2; T59, T940, T810: Scottish blood donors infected with HCV type 2; T40, T38, T36, T26, T1787: Scottish blood donors infected with HCV type 3; and Figure 10(b) shows the derivation of consensus sequences for HCV types 3, 2 and 1 oligopeptide series. Differences from consensus shown in bold. Amino acid codes: A: alanine; R: arginine; N: asparagine; D: aspartic acid; C: cysteine; Q: glutamine; E: glutamic acid; G: glycine; H: histidine; I:isoleucine; L: leucine; K: lysine; M: methionine; F: phenylalanine; P: proline; S: serine; T: threonine; W: tryptophan; Y:tyrosine; V: valine; ".": not determined.

Figures 11(a) to 11(c) show amino acid sequences of nonameric oligopeptides used for epitope mapping, derived from consensus HCV type 3, type 2 and type 1 sequences respectively. Amino acid codes: A: alanine; R: arginine; N: asparagine; D: aspartic acid; C: cysteine; Q:

For clarity, only non-identical sequences are shown in tree; e.g. Sequence 1 corresponds to those found in samples Eg-16 and Eg-29 etc. (Figure 1). Hollow squares are published sequences from Zaire; Hollow small circles are sequences from South Africa; Hollow small solid circles are sequences obtained elsewhere in the world.

Figure 15 A/B is a comparison of nucleotide (A) and amino acid (B) sequences in the core region. Symbols as for Figure 13. Single letter amino acid codes are used;

Figure 16 is a phylogenetic analysis of part of the core region using the maximum likelihood algorithm, shown as an unrooted tree. Sequences are numbered as in Figure 14; sequence 30 is that of HC-J8 (Okamato et al. Virology 188:331 -341); and

Figure 17 shows cleavage patterns for A) HaeIII/RsaI and B) ScrFI in 5'NCR.

I) ANALYSIS OF HEPATITIS C VIRUS AND PHYLOGENETIC RELATIONSHIP OF TYPES 1, 2 and 3

Introduction

Sequence analysis of the 5' non coding region of hapatitis C virus (HCV) amplified from the plasma of individuals infected in Britain revealed the existence of three distinct groups of HCV, differing by 9-14% in nucleotide sequence. Two of the groups identified were similar to those of HCV variants previously termed type 1 and type 2, while the third group appeared to represent a

possibility that mismatches between the primers and the variant sequence will prevent amplification. We have used several strategies to overcome this problem. For initial virus detection, we used primers in the 5'NCR, which are reported to highly conserved amongst type 1 variants (4, 11, 13, 16, 23, 24, 26, 33), and between K1 and K2 (23). Sequence analysis of the blood donors allowed the identification of type 1 and type 2 variants by comparison with published sequence data. This analysis also revealed the existence of a third "type" of HCV that appeared to be as distinct from type 1 as type 2 was (Figs. 1,2; Table 3). Based on our initial tentative classification, we sought corroboration of our findings in other (coding) and more variable regions of the viral genome.

Analysis of the NS-5 region, which was based on several sequences of each of the three types (Figs. 3, 4; Table 3), confirmed the existence of 3 major groups, with type 3 sequences forming a relatively homogeneous group that was quite distinct from types 1 and 2. The proposed separation of type 1 sequences into PT and K1 "sub-types" and type 2 sequences into K2a and K2b is supported by this analysis, in which the single type 2 blood donor sequence obtained in this study appears most similar to K2b. Differentiation of HCV type 1 sequences into two groups is also clearly shown in the core (Fig.7) and NS-3 regions (Fig.5), in both cases with the type 3 sequences appearing considerably more distant.

reading frame encoding a polyprotein that is subsequently cleaved into structural and non-structural proteins. Weak sequence homologies have been detected with several other virus groups that have positive-sense RNA genomes (19,21). Although the overall degree of sequence dissimilarity between types 1, 2 and 3 cannot be measured by comparison of the small regions of sequence analysed in this study, a rough estimate of the extent of divergence in protein coding regions is given by an examination of the divergence of the partial core sequence. This shows that the difference between HCV type 1 and type 3 core region (approximately 10% amino acid sequence divergence) is comparable to that which exists between different serotypes of the flavivirus, tick-borne encephalitis virus (14%; ref.20), but lower than that which is found between serotypes of a mosquito borne flavivirus, dengue fever virus (33%), and the West Nile (WN) subgroup (28-43% divergence). The 5'NCR sequences of the different members of WN subgroup are also considerably more diverse than those of the three types of HCV (=50% similarity; ref.5), although within each of the members e.g. Murray Valley encephalitis virus, the 5'NCR is extremely well conserved (>95% similarity; ref.5). On the basis of these analogies, we speculate that the major types of HCV represent distinct "serotypes", each capable of human infection irrespective of the immune response mounted against other HCV types.

transcriptase (Promega) in 20ul buffer containing 50 mM Tris-HCl (pH 8.0), 5mM $MgCl_2$, 5 mM dithiothreitol, 50 mM KCl, 0.05 ug/ul BSA, 15% DMSO, 600 uM each of dATP, dCTP, dGTP and TTP, 1.5 uM primer and 10 U RNAsin (Promega).

PCR was performed from 1 ul of the cDNA over 25 cycles with each consisting of 25 sec. at 94°C, 35 sec. at 50°C and 2.5 min. at 68°C. The extension time for the last cycle was increased to 9.5 min. The reactions were carried out with 0.4 unit Taq polymerase (Northumbria Biologicals Ltd.) in 20 ul buffer containing 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 33 uM each of dATP, dCTP, dGTP and dTTP and 0.5 uM of each of the outer nested primers. One ul of the reaction mixture was then transferred to a second tube containing the same medium but with the inner pair of nested primers, and a further 25 heat cycles were carried out with the same programme. The PCR products were electrophoresed in 3% low melting point agarose gel (IBI) and the fragments were detected by ethidium bromide staining and UV illumination. For sequence analysis, single molecules of cDNA were obtained at a suitable limiting dilution at which a Poisson distribution of positive and negative results was obtained (30).

Direct Sequencing of PCR Products. The PCR products were purified by glass-milk extraction ("GeneClean"; Bio101, Inc.). One quarter of the purified products was used in

To establish the interrelationships of the major types of HCV, we have separately analysed several regions of the viral genome that differ in sequence variability and evolutionary constraint. Thus the conclusions drawn from the sequence comparisons are not subject to spurious evolutionary phenomena that may affect a particular region. However, one problem with the analysis presented here was the absence of a viral sequence that was sufficiently distantly related to HCV to serve as an out-group. Thus, although we describe the interrelationships of different sequence variants of HCV, it should be stressed that we have no means of deciding which sequence is ancestral to the others. The trees are thus drawn in the less familiar un-rooted form to indicate this.

RESULTS

1) Analysis of the 5' non-coding region. Samples were obtained from 18 blood donors that were repeatedly reactive in the Abbott 2nd Generation enzyme immunoassay and which were confirmed or indeterminate in the Chiron 4-RIBA (E-b1 through E-b18, ref.10). HCV sequences present in stored plasma samples from each donor were amplified with primers corresponding to sites in the 5'NCR (12,25) that are well conserved between all known HCV type 1 and type 2 variants (4,11,13,16,23,24,26,33). Sequencing of the PCR product, after limiting dilution to

2) Analysis of the NS-5 Region. The nucleotide sequence of the NS-5 region has been found to vary significantly between the previously described K1 and K2 variants of HCV (7). To investigate whether type 3 sequences were equally distant from the other two types in this region as well as in the 5'NCR, we compared sequences from four type 3 blood donors (E-b1, E-b2, E-b3 and E-b7) and one type 2 donor (E-b12) with previously published sequences (Fig. 3; Fig.4; table 3).

A remarkable variation was observed between sequences of the three types in this region. Again, type 3 sequences form a separate group from type 1 and type 2 in this region. However, unlike the 5'NCR, there appear to be subdivisions within the type 1 and type 2 groups. Type 1 sequences are split between those found in Japanese infected individuals (e.g. HCV-J; HCV-BK; sequence numbers 12, 13, 16-20 in table 2) and those of U.S.A. origin (HCV-1, Pt-1, H77, H90; sequence numbers 1-4; fig.4). There is also some evidence for a split between type 2 sequences, those corresponding to their previous designation as K2a (7) appearing distinct from type K2b sequences and the Scottish blood donor, E-b12.

Table 3 shows that the average nucleotide distances between the two groups of HCV type 1 sequences is 25% (indicated here as type 1a [USA] and type 1b [Japanese]), with variation of only 4-7% within each group. The nucleotide sequence divergence within the two type 1

These sequences are representative of the range of variation found in this region in individuals infected in Britain; comparison of the tree previously published (31) with fig.6 shows that the former forms a very small component of the overall tree obtained once Japanese type 1 and type 3 sequences are added.

The maximum likelihood tree shows that type 1 and type 3 have diverged considerably from each other. As was found in the NS-5 region, subtypes of type 1 sequences are found in NS-3. Again, sequences of Japanese origin (HCV-J, HCV-BK and JH) are distinct from the prototype (PT) sequence, and those found in Scottish blood donors (E-b16, E-b17, p1-3), IVDUs (i1-5) and haemophiliacs (h1-5), all of which correspond to the prototype sequence (fig.5). However, the average subtype difference (23%) is lower than those that exist between HCV-1 and HCV-J with the four type 3 sequences (37-43%). As reported previously (31), the majority of nucleotide substitutions that exist between type 1 sequences are silent (i.e.do not affect the encoded amino acid sequence), while numerous amino acid substitutions exist between type 1 and type 3 sequences (fig.5). The analysis of the NS-3 region includes the sequence of clone A (35) which was obtained from Japanese patients with NANB hepatitis, and which was reported to be distinct from existing HCV type 1 sequences. In Fig. 6, this sequence appears to be distinct from both HCV type 1 and type 3, with corrected

TABLE 1

SEQUENCES AND SOURCES OF PRIMERS USED FOR AMPLIFICATION OF HCV GENOME.

Name	Region	Position of 5'base[a]	Sense[b]	Sequences 5'-3'	Ref.
209	5'NCR	8	-	ATACTCGAGGTGCACGGTCTACGAGACCT	(12)
211	5'NCR	-29	-	CACTCTCGAGCACCCTATCAGGCAGT	(12)
939	5'NCR	-297	+	CTGTGAGGAACTACTGTCTT	(25)
940	5'NCR	-279	+	TTCACGCAGAAAGCGTCTAG	(25)
410	CORE	410	-	ATGTACCCCATGAGGTCGGC	
406	CORE	-21	+	AGGTCTCGTAGACCGTGCATCATGAGCAC	
288	NS-3	4951	-	CCGGCATGCATGTCATGATGTAT	(31)
290	NS-3	4932	-	GTATTTGGTGACTGGGTGCGTC	(31)
208	NS-3	4662	+	TCTTGAATTTTGGGAGGGCGTCTT	
207	NS-3	4699	+	CATATAGATGCCCACTTCCTATC	
007	NS-4	5293	-	AACTCGAGTATCCCACTGATGAAGTTCCACAT	
220	NS-4	5278	-	CACATGTGCTTCGCCCAGAA	
HCV type 3:					
221	NS-4	4858	+	GGACCTACGCCCCTTCTATA	
008	NS-4	4878	+	TCGGTTGGGGCCTGTCCAAAATG	
HCV type 2:					
281	NS-4	4858	+	GGTCCCACCCCTCTCCTGTA	
509	NS-4	4878	+	CCGCTTGGGTTCCGTTACCAACG	
HCV type 1:					
253	NS-4	4858	+	GGGCCAACACCCCTGCTATA	
196	NS-4	4878	+	CAGACTGGGCGCCGTTCAGAATG	

TABLE 2

SOURCE AND CITATION OF PREVIOUSLY PUBLISHED HCV SEQUENCES USED IN THIS STUDY

No.	Type	Abbreviation	Geographical Source	Reference	Ref. No.
1	1	HCV-1	U.S.A.	Choo *et al.*, 1991	(4)
2	1	Pt-1	Japan	Nakao *et al.*, 1991	(23)
				Enomoto *et al.*, 1990	(7)
3, 4	1	H77, H90	U.S.A.	Ogata *et al.*, 1991	(24)
5, 6	1	GM-1, GM-2	Germany	Fuchs *et al.*, 1991	(11)
7	1	J1	Japan	Han *et al.*, 1991	(13)
8	1	A1	Australia	Han *et al.*, 1991	(13)
9	1	S1	S. Africa	Han *et al.*, 1991	(13)
10	1	T1	Taiwan	Han *et al.*, 1991	(13)
11	1	U18/I24	U.S.A/Italy	Han *et al.*, 1991	(13)
12	1	HCV-J	Japan	Kato *et al.*, 1990	(16)
13	1	HCV-BK	Japan	Takamizawa *et al.*, 1991	(33)
14,15	1	HC-J1, -J4	Japan	Okamoto *et al.*, 1990	(26)
16-20	1	K1, K1- 1-4	Japan	Enomoto *et al.*, 1990	(7)
21	1	JH	Japan	Kubo *et al.*, 1990	(17)
22	1	J7	Japan	Takeuchi *et al.*, 1990	(34)
23-26	2	K2a, K2a-1,	Japan	Nakao *et al.*, 1991	(23)
		K2b, K2b-1		Enomoto *et al.*, 1990	(7)
27	?	Clone A	Japan	Tsukiyama-Kohara, 1991	(35)

II). SEROLOGICAL REACTIVITY OF BLOOD DONORS INFECTED WITH THREE DIFFERENT TYPES OF HEPATITIS C VIRUS.

HCV sequences were amplified in the 5'non-coding region (5'NCR), core, NS-3 and NS-5 regions from blood donors, haemophiliacs and intravenous drug abusers.

Blood donations that were repeatedly reactive on screening with Abbott 2nd generation enzyme immunoassay (EIA) and positive or indeterminate by Ortho recombinant immunoblot assay (RIBA) were amplified by primers in the 5'NCR (reference 10). The first fourteen PCR-positive blood donations (where PCR was used to amplify and thus detect HCV RNA present in the blood) were then typed by sequence analysis of the amplified region, and compared with their serological reactivity to a range of structural and non-structural peptides in two 1st generation EIAs (Ortho HCV ELISA; Abbott HCV EIA) and two RIBA assays (Ortho RIBA and Innogenetics LIA; Table 4). The five donations containing HCV type 1 sequences were positive in both EIAs, reacted with all antigens in the Ortho RIBA assay, and were broadly reactive in the LIA. However, all but two of the sera from donors with type 2 and 3 infections were completely negative on anti-C100 EIA screening and failed to react with 5-1-1, C100 (RIBA) and NS4 (LIA).

TABLE 4

SEROLOGICAL REACTIVITY OF SERA FROM BLOOD DONORS INFECTED WITH THREE TYPES OF HEPATITIS C VIRUS

Donor Number	HCV genotype	anti-C100 O*	anti-C100 A†	Ortho RIBA 5-1-1	Ortho RIBA C100	Ortho RIBA C33	Ortho RIBA C22	Innogenetics LIA NS4	Innogenetics LIA NS5	Innogenetics LIA C1‡	Innogenetics LIA C2	Innogenetics LIA C3	Innogenetics LIA C4
E-b13	1	+	+	3§	4	4	4	2§	3	1	2	1	1
E-b15		+	+	4	4	4	4	2	3	3	2	2	1
E-b16		+	+	4	4	4	4	2	3	2	3	3	-
E-b17		+	+	4	4	4	4	3	3	3	2	1	1
E-b18		+	+	4	4	4	4	3	-	2	1	1	-
E-b9	2	+	+	-	1	3	4	-	-	3	1	1	3
E-b10		-	-	-	-	4	4	-	3	2	2	2	-
E-b11		-	-	-	-	4	4	-	3	4	2	2	3
E-b12		-	-	-	-	4	4	-	1	3	1	2	2
E-b1	3	-	-	-	-	-	4	-	1	3	1	-	3
E-b2		-	-	-	-	4	4	-	2	1	1	1	2
E-b3		+	+	-	-	2	4	2	2	1	2	2	1
E-b5		-	-	-	-	2	4	-	-	3	1	2	3
E-b7		-	-	-	-	-	4	-	2	3	1	1	4

*Ortho HCV ELISA (Recombinant C100-3)
†Abbott HCV ELA (Hepatitis C Recombinant DNA Antigen)
‡Core oligopeptides, 1-4
§Bands scored - (negative) to 4 (strong positive) according to manufacturers instructions.

with primers corresponding to sense and anti-sense sequences spanning the antigenic region of NS-4 (table 1). Nucleotide sequences obtained from the amplified DNA were compared and used to define consensus sequences for each HCV type. In-frame translation of the nucleotide sequences yielded an uninterrupted consensus amino acid sequence that was used to define a series of overlapping oligopeptides for epitope mapping.

Epitope mapping and determination of antibody specificities

Overlapping synthetic peptides were synthesised on polypropylene pins using kits commercially available from Cambridge Research Biochemicals Ltd. The principle of the addition reactions is described in refs (Geysen et al., 1984; Geysen et al., 1985). Antibody reactions were carried out on pins disrupted by sonication (30 minutes) in 1% sodium dodecyl sulphate, 0.1% 2-mercaptoethanol, 0.1M sodium dihydrogen orthophosphate. Pins were pre-coated in 1% ovalbumin, 1% bovine serum albumin, 0.1% Tween-20 in phosphate buffered saline (PBS) for one hour at room temperature. Serum or plasma was diluted 1.40 in PBS + 0.1% Tween-20 (PBST) and incubated with the blocked pins at 4°C for 18 hours. After washing in 4 changes of PBST (10 minutes at room temperature, with agitation), bound antibody was detected by incubation in a 1/20000 dilution of affinity isolated anti-human IgG, peroxidase conjugate (Sigma) for one hour at room temperature.

considerably from the amino acid sequences of other blood donors infected with HCV types 1 and 2 (T16, 42, 77, 1801, 1825, 351, 940 and 810; Figure 6a). Sequence comparison between the major HCV types from residues 1679 to 1769 reveals three regions of considerable amino acid sequence variability. Most of the observed differences between types involve non-synonymous amino acid substitutions, particularly alternation of acidic and basic residues in the hydrophillic regions. These changes would be expected to profoundly alter the overall conformation of the protein, and its antigenicity.

The consensus amino acid sequences in this region of types 1-3 (Figure 6b) were used to define three series of 82 nonameric oligopeptides overlapping by eight of the nine residues with those before and after in the series (Figure 7a-c). These were synthesised on a 12 x 8 arrays of polypropylene pins as described in Methods. Antibody reactivity to the immobilised antigens on the pins was determined by indirect ELISA, using an overnight incubation with a 1/40 dilution of test serum overnight at 4°C, followed by washing, and detection with an anti-human IgG-peroxidase conjugate and appropriate substrate (see Methods).

Reactivity of an anti-HCV negative, PCR-negative donor, with no known risk factors for HCV infection with the three series of peptides was determined. No significant reactivity is shown with any of the

exceeds that found in the core, NS-3 or NS-5 regions previously analysed (Chan et al, 1992). The function of the protein encoded by this region of the HCV genome is unknown, and the consequences of this variability on virus replication and pathogenesis are unknown. The function of the NS-4 region in flaviviruses and pestiviruses is also poorly defined.

The degree of amino acid sequence variability, and the nature of the amino acid substitutions indicate that the major sites of antibody reactivity are also those of antigenic variability. This undoubtedly underlies the restricted cross-reactivity of HCV type 1 NS-4 encoded antigens with sera from individuals infected with different HCV types. Serological diagnosis of infection is currently based entirely on recombinant or synthetic oligopeptide sequences derived ultimately from HCV type 1 sequences (Choo et al., 1991). The serological response to infection is often very restricted in its initial stages, with antibody to only one of the recombinant antigens used for screening. Not only does this present difficulties with supplementary antibody tests, where reactivity to two HCV-encoded antigens is required for confirmation, but can lead to an increased probability of failing to detect early infection with HCV types 2 and 3.

Table 7 relates HCV typing determined by PCR to results obtained using type-specific antigens (TSA) and shows good correlation for HCV1-3 types.

TABLE 6

SEQUENCES OF OLIGONUCLEOTIDES SUITABLE FOR DIRECT DETECTION OF HCV TYPE 3 IN CLINICAL SPECIMENS BY POLYMERASE CHAIN REACTION

Name	Region	Position of 5'base*	Pol.†	Sequences 5'-3'‡
007	NS-4	5293	-	AACTCGAGTATCCCACTGATGAAGTTCCACAT
220	NS-4	5278	-	CACATGTGCTTCGCCCAGAA
Type 3:¶				
TS-3a	NS-4	5140	+	GCCGCCCCATATATCGAACA
TS-3b	NS-4	5161	+	GCTCAGGTAATAGCCCACCA
Type 2:				
TS-2a	NS-4	5140	+	AAAGCCGCCCTCATTGAGGA
TS-2b	NS-4	5161	+	GGGCAGCGGATGGCGGAGAT
Type 1:				
TS-1a	NS-4	5140	+	CACTTACCGTACATCGAGCA
TS-1b	NS-4	5161	+	GGGATGATGCTCGCCGAGCA

* Position of 5'base relative to HCV genomic sequence in Choo *et al.*, (1991).
† Orientation of primer sequence (+: sense; -: anti-sense)
‡ Abbreviations: A: adenine, C: cytidine; G: guanidine, T: thymidine.
¶ Type-specific sense primers for amplification of HCV types 3, 2 and 1 variants.

PART IV IDENTIFICATION OF HCV TYPE -4

Introduction

Investigations were carried out on sequence variations in the 5' non-coding region (5'NCR) of HCV samples from a variety of worldwide geographical locations (Figure 13), and also in the core region (Figures 15A and 15B). Phylogenetic analysis (Figures 14 and 16) revealed a new distinct HCV type which we refer to herein as HCV-4.

Methods

Samples. RNA was extracted from plasma samples that were repeatedly reactive on second generation screening assays for HCV, and which were either confirmed (significant reactivity with two or more antigens in the Chiron recombinant immunoblot assay; Chiron Corporation, Emeryville, California, USA) or indeterminate (reactive with only one antigen) from blood donors and patients with NANBH. Most of the samples containing sequences that differed substantially from known HCV types came from Egypt (EG 1-33). Others came from Holland (NL-26), Hong Kong (HK 1-4), Iraq (IQ-48) and XX (xx-(6).

Sequence determination. HCV sequences were reverse transcribed and amplified with primers matching conserved regions in the 5'NCR as previously described [1]. For analysis of the core region, RNA was reverse transcribed

Results

Divergent 5'NCR sequences. Several sequences in the 5'NC region detected in samples of blood donors from Saudi Arabia, Holland and Hong Kong, and from NANBH patients in Iraq and xxx differed substantially from those found in Scottish blood donors and those reported elsewhere (Figure 13). Instead of showing the well characterised nucleotide substitutions that distinguish HCV types 1, 2 and 3 from each other, a new set of sequence differences were observed in the new variants that appeared to place them outside the existing system of virus classification. This can be more simply represented by reconstructing a phylogeny of the sequences and presenting the results as an evolutionary tree (Figure 14). This analysis confirms that sequences 1-10 cluster separately from the variants previously typed as 1,2 and 3. For convenience we will refer to sequences within this new group as type HCV type 4. Mean distances within type 4 and between type 4 and the other HCV types in the 5'NCR were comparable to those previously described for type 1-3. Although sequences within type 4 are relatively closely grouped, sequences 11, 12 and 13 differ considerably from any of the known types.

Using this phylogenetic tree, it can be seen that the majority of previously published 5'NCR sequences can be readily identified as types 1, 2 or 3. Furthermore, almost all of the sequences from Zaire (shown as hollow

PART V HCV TYPING

Introduction

In view of the sequence variations between HCV types 1,2,3 and 4 differences in restriction enzyme cleavage sites exist, leading to different endonuclease cleavage patterns. This technique was used to identify HCV genotypes in blood samples from a variety of sources worldwide.

(A) Typing of HCV1-3

METHODS

Serum Samples: Samples from blood donors in six countries, Scotland, Finland, Netherlands, Hong Kong and Australia and Japan, were available from routine 2nd Generation anti-HCV ELISA screening (Ortho or Abbott). Donor samples that were repeatedly reactive in the above tests were further investigated using a supplementary test (Ortho RIBA: Finland, Netherlands, Australia, Egypt, Abbott Matrix: Hong Kong) or samples were titred for anti-HCV by ELISA (Japan). Samples that were positive (significant reactivity with two or more HCV antigens (1+ to 4+) or indeterminate (reactivity with one antigen only) in the RIBA test or had a titre of > X 4096 by ELISA (Japan only) were tested for viral RNA by Polymerase Chain Reaction (PCR).

RNA PCR: PCR for the detection of HCV RNA was carried out as previously described by Chan et al (reference 1a) using primers in the 5'non-coding region (5'NCR) in a nested PCR, with primers 209/939 and 211/940(5) in first and second reactions respectively.

TABLE 8

PREVALENCE OF HCV TYPES IN DIFFERENT COUNTRIES

COUNTRY	HCV TYPES (%)		
	HCV-1	HCV-2	HCV-3
Scotland	86(51%)	21(13%)	60(36%)
Finland	3(25%)	5(42%)	4(33%)
Netherlands	18(60%)	7(23%)	5(17%)
Hong Kong	22(63%)	0(0%)	0(0%)
Australia	13(57%)	3(13%)	7(30%)
Japan	31(77%)	9(23%)	0(0%)
Egypt	0(0%)	0(0%)	0(0%)

PCR and typing. RNA was reverse transcribed with primer 940 and cDNA amplified in a two stage nested PCR reaction with primers 940/939, followed by 209/211 as previously described (Chan et al. 1992). PCR product was radiolabelled with [^{35}S]-dATP analysed by restriction endonuclease cleavage (McOmish et al. Transfusion, 32:no.11 1992). Samples were cleaved with ScrFI and a combination of HaeIII/RsaI in two separate reactions to identify HCV types 1/4, 2, 3. Figure 17 shows endonuclease cleavage patterns. HCV types 1 and 4 were differentiated by a third reaction with HinfI (see Results). Two samples yielded restriction patterns that were different from those of the four known types of HCV and were analysed further by direct sequence analysis of the amplified DNA (Chan et al. 1992). These two samples contained 5'NCR sequences distinct from those of known HCV types and currently remain unclassified.

RESULTS

Modification of RFLP method to identify HCV type 4.

Previous sequence analysis in the 5'NCR of HCV amplified from plasma of Egyptian blood donors revealed a relatively homogeneous group of novel sequence variants in both the 5'NCR and core region which were as distinct from HCV types 1, 2 and 3 as these latter types were from each other (see previous submission). This new group was designated as HCV type 4.

initially carried out with HaeIII/RsaI and ScrF1, and allowed the identification of 10 type 2 and 10 type 3 variants (Table 10). Samples showing electrophoretic patterns aA/B or bA/B were further analysed by cleavage with hinfI, yielding 38 samples with pattern a, thus identified as type 1, 22 with pattern b and 2 with pattern c, both identified as type 4. Finally, two samples showed the unusual cleavage patterns h and i with HaeIII/RsaI and pattern b with HinfI, and were therefore directly sequenced. These two sequences were similar to each other but were unlike any of the known HCV types, and also distinct from EG-28, the other sequence showing pattern i with HaeIII/RsaI (Table 10). As they cannot be currently classified, they will be referred to as type U.

TABLE 10

IDENTIFICATION OF HCV TYPES 1-4 IN STUDY SUBJECTS BY RFLP ANALYSIS OF 5'NCR SEQUENCES WITH *RsaI*/*HaeIII*, *ScrFI* AND *HinfI*

Observed cleavage pattern			Inferred HCV type				
HaeIII /*RsaI*	*ScrFI*	*HinfI*	1	2	3	4	U*
a	A/B	a	2	-	-	-	-
b	A/B	a	36	-	-	-	-
a	A/B	b	-	-	-	16	-
a	A/B	c	-	-	-	2	-
b	A/B	b	-	-	-	6	-
c	D	n.d.	-	7	-	-	-
d	E	n.d.	-	3	-	-	-
f	G	n.d	-	-	7	-	-
g	G	n.d.	-	-	3	-	-
h	A/B	b	-	-	-	-	1
i	A/B	b	-	-	-	-	1
		TOTALS	38	10	10	24	2

* Two samples yielded unusual restriction patterns with *HaeIII*/*RsaI* (h, i). Sequence analysis of the 5'NCR placed them outside existing HCV classification (samples IQ-48, EG-96).

as bacterial, yeast, mammalian and insect cells. Particular examples of such cells are E.coli, S.cerevisiae, P.pastoris, Chinese hamster ovary and mouse cells, and Spodoptera frugiperda and Tricoplusia ni. The choice of host cell may depend on a number of factors but, if post-translational modification of the HCV viral peptide is important, then an eukaryotic host would be preferred.

The present invention also provides a process for preparing a peptide as defined herein which comprises isolating the DNA sequence, as herein defined, from the HCV genome, or synthesising DNA sequence encoding the peptides as defined herein, or generating a DNA sequence encoding the peptide, inserting the DNA sequence into an expression vector such that it is capable, in an appropriate host, of being expressed; transforming host cells with the expression vector, culturing the transformed host cells, and isolating the peptide.

The DNA sequence encoding the peptide may be synthesised using standard procedures (Gait, Oligonucleotide Synthesis: A Practical Approach, 1984, Oxford, IRL Press).

The desired DNA sequence obtained as described above may be inserted into an expression vector using known and standard techniques. The expression vector is normally cut using restriction enzymes and the DNA sequence inserted using blunt-end or staggered-end ligation. The

response, into a mammalian host, such as a mouse, rat, sheep or rabbit, and recovering the antibody thus produced. The peptide is generally administered in the form of an injectable formulation in which the peptide is admixed with a physiologically acceptable diluent. Adjuvants, such as Freund's complete adjuvant (FCA) or Freund's incomplete adjuvant (FIA), may be included in the formulation. The formulation is normally injected into the host over a suitable period of time, plasma samples being taken at appropriate intervals for assay for anti-HCV viral antibody. When an appropriate level of activity is obtained, the host is bled. Antibody is then extracted and purified from the blood plasma using standard procedures, for example, by protein A or ion-exchange chromatography.

Monoclonal antibody against a peptide of the present invention may be obtained by fusing cells of an immortalising cell line with cells which produce antibody against the viral or topographically related peptide, and culturing the fused immortalised cell line. Typically, a non-human mammalian host, such as a mouse or rat, is inoculated with the peptide. After sufficient time has elapsed for the host to mount an antibody response, antibody producing cells, such as the splenocytes, are removed. Cells of an immortalising cell line, such as a mouse or rat myeloma cell line, are fused with the antibody producing cells and the resulting fusions screened to identify a cell line, such as a hybridoma,

infection, or the response to a particular viral antigen may be associated with clearance of the virus. Thus the exact approach adopted for the diagnosis of a viral infection depends upon the particular circumstances and the information sought. In the case of HCV, a diagnostic assay may embody any one of these three approaches.

In an assay for the diagnosis of HCV involving detection of viral nucleic acid, the method may comprise hybridising viral RNA present in a test sample, or cDNA synthesised from such viral RNA, with a DNA sequence corresponding to the nucleotide sequences of the present invention or encoding a peptide of the invention, and screening the resulting nucleic acid hybrids to identify any HCV viral nucleic acid. The application of this method is usually restricted to a test sample of an appropriate tissue, such as a liver biopsy, in which the viral RNA is likely to be present at a high level. The DNA sequence corresponding to a nucleotide sequence of the present invention or encoding a peptide of the invention may take the form of an oligonucleotide or a cDNA sequence optionally contained within a plasmid. Screening of the nucleic acid hybrids is preferably carried out by using a labelled DNA sequence. Preferably the peptide of the present invention is part of an oligonucleotide wherein the label is situated at a sufficient distance from the peptide so that binding of the peptide to the viral nucleic acid is not interfered with by virtue of the label

taken from any of the appropriate tissues and physiological fluids mentioned above for the detection of viral nucleic acid. If a physiological fluid is obtained, it may optionally be concentrated for any viral antigen or antibody present.

A variety of assay formats may be employed. The peptide can be used to capture selectively antibody against HCV from solution, to label selectively the antibody already captured, or both to capture and label the antibody. In addition, the peptide may be used in a variety of homogeneous assay formats in which the antibody reactive with the peptide is detected in solution with no separation of phases.

The types of assay in which the peptide is used to capture antibody from solution involve immobilization of the peptide on to a solid surface. This surface should be capable of being washed in some way. Examples of suitable surfaces include polymers or various types (moulded into microtitre wells; beads; dipsticks of various types; aspiration tips; electrodes; and optical devices), particles (for example latex; stabilized red blood cells; bacterial or fungal cells; spores; gold or other metallic or metal-containing sols; and proteinaceous colloids) with the usual size of the particle being from 0.02 to 5 microns, membranes (for example of nitrocellulose; paper; cellulose acetate; and high porosity/high surface area membranes of an organic or inorganic material).

may be achieved by use of labelled molecule or particle as described above which will react with the captured antibody (for example protein A or protein G and the like; anti-species or anti-immunoglobulin-sub-type; rheumatoid factor; or antibody to the peptide, used in a competitive or blocking fashion), or any molecule containing an epitope contained in the peptide.

The detectable signal may be optical or radioactive or physico-chemical and may be provided directly by labelling the molecule or particle with, for example, a dye, radiolabel, electroactive species, magnetically resonant species or fluorophore, or indirectly by labelling the molecule or particle with an enzyme itself capable of giving rise to a measurable change of any sort. Alternatively the detectable signal may be obtained using, for example, agglutination, or through a diffraction or birefringent effect if the surface is in the form of particles.

Assays in which a peptide itself is used to label an already captured antibody require some form of labelling of the peptide which will allow it to be detected. The labelling may be direct by chemically or passively attaching for example a radio label, magnetic resonant species, particle or enzyme label to the peptide; or indirect by attaching any form of label to a molecule which will itself react with the peptide. The chemistry of bonding a label to the peptide can be directly through

magnetic resonance or enzyme measurement). Addition of either viral peptide or antibody in a sample results in restriction of the interaction of the labelled pair and thus in a different level of signal in the detector.

A suitable assay format for detecting HCV antibody is the direct sandwich enzyme immunoassay (EIA) format. A peptide is coated onto microtitre wells. A test sample and a peptide to which an enzyme is coupled are added simultaneously. Any HCV antibody present in the test sample binds both to the peptide coating the well and to the enzyme-coupled peptide. Typically, the same peptide are used on both sides of the sandwich. After washing, bound enzyme is detected using a specific substrate involving a colour change. A test kit for use in such an EIA comprises:

(1) a peptide, as herein defined labelled with an enzyme;

(2) a substrate for the enzyme;

(3) means providing a surface on which a peptide is immobilised; and

(4) optionally, washing solutions and/or buffers.

It is also possible to use IgG/IgM antibody capture ELISA wherein an antihuman antibody is coated onto microlitre wells, a test sample is added to the well. Any IgG or IgM antibody present in the test sample will then bind to the anti-human antibody. A peptide of the present invention, which has been labelled, is added to the well and the peptide will bind to any IgG or IgM antibody which

other HCV genotypes. Accordingly, it is clearly desirable to supplement testing for HIV-1 with testing for all other genotypes, for example, types 2, 3 and 4, and also any further genotypes that may be discovered.

To test for a spectrum of genotypes, there may be provided a series of assay means each comprising one or more antigenic peptides from one genotype of HCV, for example, a series of wells in a microtitre plate, or an equivalent series using the bead format. Such an assay format may be used to determine the genotype of HCV present in a sample. Alternatively, or in addition, an assay means may comprise antigenic peptides from more than one genotype, for example, a microwell or bead may be coated with peptides from more than one genotype.

It has been found advantageous to use more than one HCV antigen for testing, in particular, a combination comprising at least one antigenic peptide derived from the structural region of the genome and at least one antigenic peptide derived from the non-structural region, especially a combination of a core antigen and at least one antigen selected from the NS3, NS4 and NS5 regions. The wells or beads may be coated with the antigens individually. It has been found advantageous, however, to fuse two or more antigenic peptides as a single polypeptide, preferably as a recombinant fusion polypeptide. Advantages of such an approach are that the individual antigens can be combined in a fixed, predetermined ratio (usually equimolar) and

The peptide of the present invention may be incorporated into a vaccine formulation for inducing immunity to HCV in man. For this purpose the peptide may be presented in association with a pharmaceutically acceptable carrier.

For use in a vaccine formulation, the peptide may optionally be presented as part of an hepatitis B core fusion particle, as described in Clarke et al (Nature, 1987, 330, 381-384), or a polylysine based polymer, as described in Tam (PNAS, 1988, 85, 5409-5413). Alternatively, the peptide may optionally be attached to a particulate structure, such as lipsomes or ISCOMS.

Pharmaceutically acceptable carriers include liquid media suitable for use as vehicles to introduce the peptide into a patient. An example of such liquid media is saline solution. The peptide may be dissolved or suspended as a solid in the carrier.

The vaccine formulation may also contain an adjuvant for stimulating the immune response and thereby enhancing the effect of the vaccine. Examples of adjuvants include aluminium hydroxide and aluminium phosphate.

The vaccine formulation may contain a final concentration of peptide in the range from 0.01 to 5 mg/ml, preferably from 0.03 to 2 mg/ml. The vaccine formulation may be incorporaterd into a sterile container, which is then sealed and stored at a low temperature, for example 4°C, or may be freeze-dried.

LITERATURE CITED

1a. Chan. S-W. McOmish. F. Holmes. EC. Dow. B. Peutherer. JF. Follett. E. Yap. PL and Simmonds. P (1992). *J Gen Virol*: 73:1131-1141.

1. Chan. S.-W., P. Simmonds. F. McOmish. P.-L. Yap. R. Mitchell. B. Dow. and E. Follett. 1991. Serological reactivity of blood donors infected with three different types of hepatitis C virus. Lancet 338.: 1391.

2. Chomczynski. P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162:156-159.

3. Choo. Q.L., G. Kuo. A.J. Weiner. L.R. Overby. D.W. Bradley, and M. Houghton. 1989. Isolation of a cDNA derived from a blood-borne non-A. non-B hepatitis genome. Science 244:359-362.

4. Choo. Q.L., K.H. Richman. J.H. Han. K. Berger. C. Lee. C. Dong. C. Gallegos. D. Coit. R. Medina Selby. P.J. Barr. A.J. Weiner. D.W. Bradley. G. Kuo. and M. Houghton. 1991. Genetic organization and diversity of the hepatitis C virus. Proc. Natl. Acad. Sci. U.S.A. 88:2451-2455.

5. Coelen. R.J. and J.S. Mackenzie. 1990. The 5' terminal non-coding region of Murray Valley encephalitis virus RNA is highly conserved. J. Gen. Virol. 71:241-245.

6. Devereux. J., P. Haeberli. and O. Smithies. 1984. Comprehensive set of sequence analysis programs for the VAX. Nucleic. Acids. Res. 12:387-395.

7. Enomoto. N., A. Takada. T. Nakao. and T. Date. 1990. There are two major types of hepatitis C virus in Japan. Biochem. Biophys. Res. Commun. 170:1021-1025.

15. **Japanese Red Cross Non-A. Non-B Hepatitis Research Group** 1991. Effect of screening for hepatitis C virus antibody and hepatitis B virus core antibody on the incidence of post-transfusion hepatitis. Lancet **338**:1040-1041.

16. **Kato. N., M. Hijikata. Y. Ootsuyama. M. Nakagawa. S. Ohkoshi. T. Sugimura. and K. Shimotohno.** 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A. non-B hepatitis. Proc. Natl. Acad. Sci. U.S.A. **87**:9524-9528.

17. **Kubo. Y.. K. Takeuchi. S. Boonmar. T. Katayama. Q.L. Choo. G. Kuo. A.J. Weiner, D.W. Bradley, M. Houghton. I. Saito. and T. Miyamura.** 1989. A cDNA fragment of hepatitis C virus isolated from an implicated donor of post-transfusion non-A. non-B hepatitis in Japan. Nucleic. Acids. Res. **17**:10367-10372.

18. **Kuo. G., Q.L. Choo. H.J. Alter. G.L. Gitnick. A.G. Redeker. R.H. Purcell. T. Miyamura. J.L. Dienstag, M.J. Alter. C.E. Stevens. G.E. Tegtmeier. F. Bonino. M. Columbo. W.-S. Lee. C. Kuo. K. Berger. J.R. Shuster. L.R. Overby, D.W. Bradley, and M. Houghton.** 1989. An assay for circulating antibodies to a major etiologic virus of human non-A. non-B hepatitis. Science **244**:362-364.

19. **Laín. S.. J.L. Reichmann. M.T. Martin. and J.A. Garcia.** 1989. Homologous potyvirus and flavivirus proteins belonging to a superfamily of helicase-like proteins. Gene **82**:357-362.

20. **Mandl. C.W.. F.X. Heinz. and C. Kunz.** 1988. Sequence of the structural proteins of tick-borne encephalitis virus (Western subtype) and comparative analysis with other flaviviruses. Virology **166**:197-205.

28. **Saitou. N. and T. Imanishi.** 1989. Relative efficiencies of the Fitch-Margoliash. maximum-parsimony,. maximum-likelihood. minimum evolution. and neighbor-joining methods of phylogenetic tree construction in obtaining the correct tree. Mol. Biol. Evol. 6:514-525.

29. **Saitou. N. and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

30. **Simmonds. P., P. Balfe, J.F. Peutherer, C.A. Ludlam. J.O. Bishop, and A.J. Leigh Brown.** 1990. Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers. J. Virol. 64:864-872.

31. **Simmonds. P., L.Q. Zhang, H.G. Watson. S. Rebus. E.D. Ferguson. P. Balfe. G.H. Leadbetter, P.L. Yap, J.F. Peutherer, and C.A. Ludlam.** 1990. Hepatitis C quantification and sequencing in blood products. haemophiliacs. and drug users. Lancet 336:1469-1472.

32. **Staden. R.** 1984. Graphic methods to determine the function of nucleic acid sequences. Nucleic. Acids. Res. 12:521-538.

33. **Takamizawa. A.. C. Mori. I. Fuke. S. Manabe. S. Murakami. J. Fujita. E. Onishi. T. Andoh. I. Yoshida. and H. Okayama.** 1991. Structure and organization of the hepatitis C virus genome isolated from human carriers. J. Virol. 65:1105-1113.

34. **Takeuchi. K.. Y. Kubo. S. Boonmar. Y. Watanabe. T. Katayama. Q.L. Choo. G. Kuo. M. Houghton. I. Saito. and T. Miyamura.** 1990. Nucleotide sequence of core and envelope genes of the hepatitis C virus genome derived directly from human healthy carriers. Nucleic. Acids. Res. 18:4626.

SEQUENCE LISTING (FIGURE 1 and 1a)

SEQ ID NO : 1

SEQUENCE TYPE : Nucleotide cDNA sequence

SEQUENCE LENGTH : 194 base pairs

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULAR TYPE : genomic DNA from HCV types 1-3

ORIGINAL SOURCE

ORGANISM : human blood samples

USE :

DEPOSIT :

FEATURES

Bases - 255 to -62 of the 5′ non-coding region showing variations in cDNA sequence between HCV types 1-3.

SEQUENCE LISTING (FIGURE 5)

SEQ ID NO : 3

SEQUENCE TYPE : deduced peptide sequence

SEQUENCE LENGTH : 57 amino acids

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULAR TYPE : HCV peptide

ORIGINAL SOURCE

ORGANISM : human blood samples

USE :

DEPOSIT :

FEATURES

Amino acids 1577 to 1633 of NS-3 region showing variations in peptide sequence between HCV types 1-3.

SEQUENCE LISTING (FIGURE 9a)

SEQ ID NO : 5

SEQUENCE TYPE : Nucleotide sequence

SEQUENCE LENGTH : 367 base pairs

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULAR TYPE : HCV genomic DNA

ORIGINAL SOURCE

ORGANISM : human blood samples

USE :

DEPOSIT :

FEATURES

Bases 4911 to 5277 of NS-4 region of HCV-3 showing individual variations and consensus sequence.

SEQUENCE LISTING (FIGURE 10a and 10b)

SEQ ID NO : 7

SEQUENCE TYPE : peptide sequence

SEQUENCE LENGTH : 90 amino acids

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULAR TYPE : HCV peptide

ORIGINAL SOURCE

ORGANISM : human blood samples

USE :

DEPOSIT :

FEATURES

Amino acids 1679 to 1768 of NS-4 region of HCV1-3 showing individual variations and consensus sequence.

SEQUENCE LISTING (FIGURE 13)

SEQ ID NO : 9

SEQUENCE TYPE : Nucleotide cDNA sequences

SEQUENCE LENGTH : 30, 70 and 32 base pairs

STRANDEDNESS : single

TOPOLOGY : linear

MOLECULAR TYPE : genomic DNA from HCV types 1 to 4

ORIGINAL SOURCE

ORGANISM : human blood samples

USE :

DEPOSIT :

FEATURES

Bases -245 to -216; -185 to -116; and -101 to -70 in the 5'NCR region of HCV types 1-4.

CLAIMS

1. A polynucleotide sequence unique to hepatitis C virus type 3 or 4 (HCV-3 or HCV-4).

2. A polynucleotide sequence according to claim 1 which is a cDNA sequence.

3. An HCV-3 or HCV-4 specific polynucleotide sequence from the core, NS3, NS4 or NS5 regions.

4. An HCV-3 or HCV-4 specific polynucleotide sequence comprised within the sequences shown in Figures 1 and 1a, 9a, 13 and 15a.

5. An HCV-3 or HCV-4 polynucleotide sequence which codes for an antigenic peptide.

6. An HCV-3 or HCV-4 polynucleotide sequence which codes for C-100, 5-1-1, C33 or C22 peptides having sequence alterations unique to HCV-3 or HCV-4.

7. An HCV-3 polynucleotide sequence encoding the polypeptide

KPALVPDKEVLYQQYDEM or the polypeptide

ECSQAAPYIEQAQVIAHQF

or polypeptides of substantially equivalent antigenicity.

16. An antigenic NS4 peptide specific to HCV-4 which comprises the HCV-4 sequence substantially from position 1691 to 1708 or the sequence substantially from position 1710 to 1728.

17. A fusion peptide which comprises at least two of the antigens of claims 9 to 16.

18. A fusion peptide according to claim 17 which comprises at least one of the antigens of claims 9 to 16, fused to ß-galactosidase, GST, trp E, or polyhedrin coding sequence.

19. A peptide according to any of claims 9 to 18 which is labelled.

20. A vaccine formulation which comprises an antigen according to any of claims 9-18.

21. Antibody to the antigenic peptide of any of claims 9 to 19.

22. An immunoassay device which comprises a solid substrate having attached thereto antigen according to any of claims 9 to 19.

23. A device according to claim 22 for HCV screening which comprises a mixture of antigens.

30. A method of in vitro HCV typing which comprises

- carrying out endonuclease digestion of an HCV-containing sample employing ScrFI or HaeIII/RsaI endonuclease; and
- comparing the restriction patterns with characteristic type-specific patterns.

31. A method according to claim 30 or 31 which further employs HinfI in a separate or combined digestion.

32. A method of in vitro HCV typing which comprises

- carrying out endonuclease digestion of an HCV-containing sample employing ScrFI endonuclease, the restriction pattern being characteristic of HCV-1, HCV-2 and HCV-3;

- carrying out endonuclease digestion employing HinfI endonuclease, the restriction pattern being characteristic of HCV-4.

33. A method of in vitro screening a sample for HCV antibodies which comprises;

- carrying out an immunoassay employing an antigenic peptide according to any of claims 1 to 19 or mixture thereof; and
- detecting any antibody - antigen complex produced.

FIGURE 1

		-255	-235	-215	-195	-175	-156
	E-b1	GGCGTTAGTA	CGAGTGTCGT	GCAGCCTCCA	GGACTCCCCC	TCCCGGGAGA	GCCATAGTGG	TCTGCGGAAC	CGGTGAGTAC	ACCGGAATCG	CTGGGGTGAC
	E-b2	...	....	....	....C.....	....	....	....	....	....	....
	E-b3	...	....	....	....C.....	....	....	....	....	....	....
	E-b4	...	....	....	....C.....	....	....	....	....	....	....
	E-b5	...	....	....	....C.....	....	....	....	....	....	....
	E-b6	...	....	....	....C.....	....	....	....	....	....	....
	E-b7		....	....	..C.C.....	....	....	....	....	....	....
	E-b8		....	....	....C.....	....	....	....	....	....	....
2	K2a	....	T.......A.	....	..C.C.....	....	....	....	....	.......T.	.C...AA...
	E-b9		T.........	A.........	..C.C.....	....	....	....	....	.......T.	.C...AA...
	E-b10		T.........	A.........	..C.C.....	....	....	....	....	.......T.	.C...AA...
	E-b11		T.........	A.........	..C.C.....	....	....	....	....	.......TA	.C..AAA...
	E-b12	...	T.........	A.........	..C.C.....	....	....	....	....	.......T.	.C..AAA...
2	K2b-1	....	T.......A.	....	..C.C.....	....	....	....	....	.......TA	.CG..AA...
	E-b13	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
	E-b14	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
	E-b15	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
	E-b16		T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
	E-b17		T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
	E-b18		T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	HCV-1	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	Pt-1	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	H77	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	H90	....	T.........	A.........	....C.....	....	....	....	....	.......T.	.CA..AC...
1	GM1	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	GM2	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	J1	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	A1	....	T.........	....	....C.....	......A...	....	....	....	.......T.	.C...AG...
1	B1	....	T.........	....	....C.....	......A...	....	....	....	.......T.	.C...A....
1	T1	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..A....
1	U18/I24	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	HCV-J	....	T......T..	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	HCV-BK	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	HC-J1	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...
1	HC-J4	....	T.........	....	....C.....	....	....	....	....	.......T.	.CA..AC...

FIGURE 1a

		-155		-135		-115		-95		-75	-62
	E-b1	CGGGTCCTTT	CTTGGAGCAA	CCCGCTCAAT	ACCCAGAAAT	TTGGGCGTGC	CCCCGCGAGA	TCACTAGCCG	AGTAGTGTTG	GGTCGCGAAA	GGCC
	E-b2	....	....	....	....	....	....	....	....	....	....
	E-b3	....	....	....	....	....	....	....	....	....	....
	E-b4	....	....	....	....	....	....	....	....	....	....
	E-b5	....	....	....	....	....	....	....	....	....	....
	E-b6	....	....	....	....	....	....	....	....	....	....
	E-b7	....	....	....	....	....	.....A....	C.........	....	....	....
	E-b8	....	......A...	....	....	....	....	....	....	....	....
2	K2a	T.........	......TA..	...A...T..	G...G.TC..	....	.....A..				
	E-b9	T.........	......TA..	...A...T..	G...G.CC..	....	.....A...	CTG.......	.....C....	...T......	....
	E-b10	T.........	......TA..	...A...T..	G...G.CC..	....	.....A...	CTG.......	.....C....	...T......	....
	E-b11	T.........	......TA..	...A...T..	GT..G.TC..	....	.....A...	CTG......T	.....C....	...T......	....
	E-b12	T.........	......TA..	...A...T..	GT..G.TC..	....	.....A...	CTG......T	.....C....	...T......	....
2	K2b-1	T.........	......TA..	...A...T..	GT..G.TC..	....	.....A..				
	E-b13	....	......T...	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
	E-b14	....	......T...	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
	E-b15	....	......T...	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
	E-b16	....	......TT..	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
	E-b17	....	......TT..	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
	E-b18	....	......T...	....	G..TG..G..	....	....	CTG.......	....	....	....
1	HCV-1	....	......T...	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
1	Pt-1	....	......TA..	....	G..TG..G..	....	.....A..				
1	H77	....	......TA..	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
1	H90	....	......TA..	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
1	GM1	....	......TA..	....	G..TG..G..	....	.....A...	CTG.......	....	....	...
1	GM2	....	......TA..	....	G..TG..G..	....	.....A...	CTG.......	....	....	...
1	J1	....	......TA..	....	G..TG..G..	.......C..	.....A..				
1	A1	....	......TA..	....	G..TG..G.C	....	.....A...	CTG.......	....	....	....
1	S1	....	......TA..	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
1	T1	....	......TA..	....	G..TG..G.C	....	.....A...	CTG.......	....	....	....
1	U18/I24	....	......TA..	....	G..TG..G..	....	.....A...	CTG.......	....	....	....
1	HCV-J	....	......T...	....	G..TG..G..	....	....	CTG.......	....	....	....
1	HCV-BK	....	......T...	....	G..TG..G..	....	....	CTG.......	....	....	....
1	HC-J1	....	......TA..	....	G..TG..G..	.......C..	.....A...	CTG.......	....	....	....
1	HC-J4	....	......T...	....	G..TG..G..	....	....	CTG.......	....	....	....

FIGURE 2

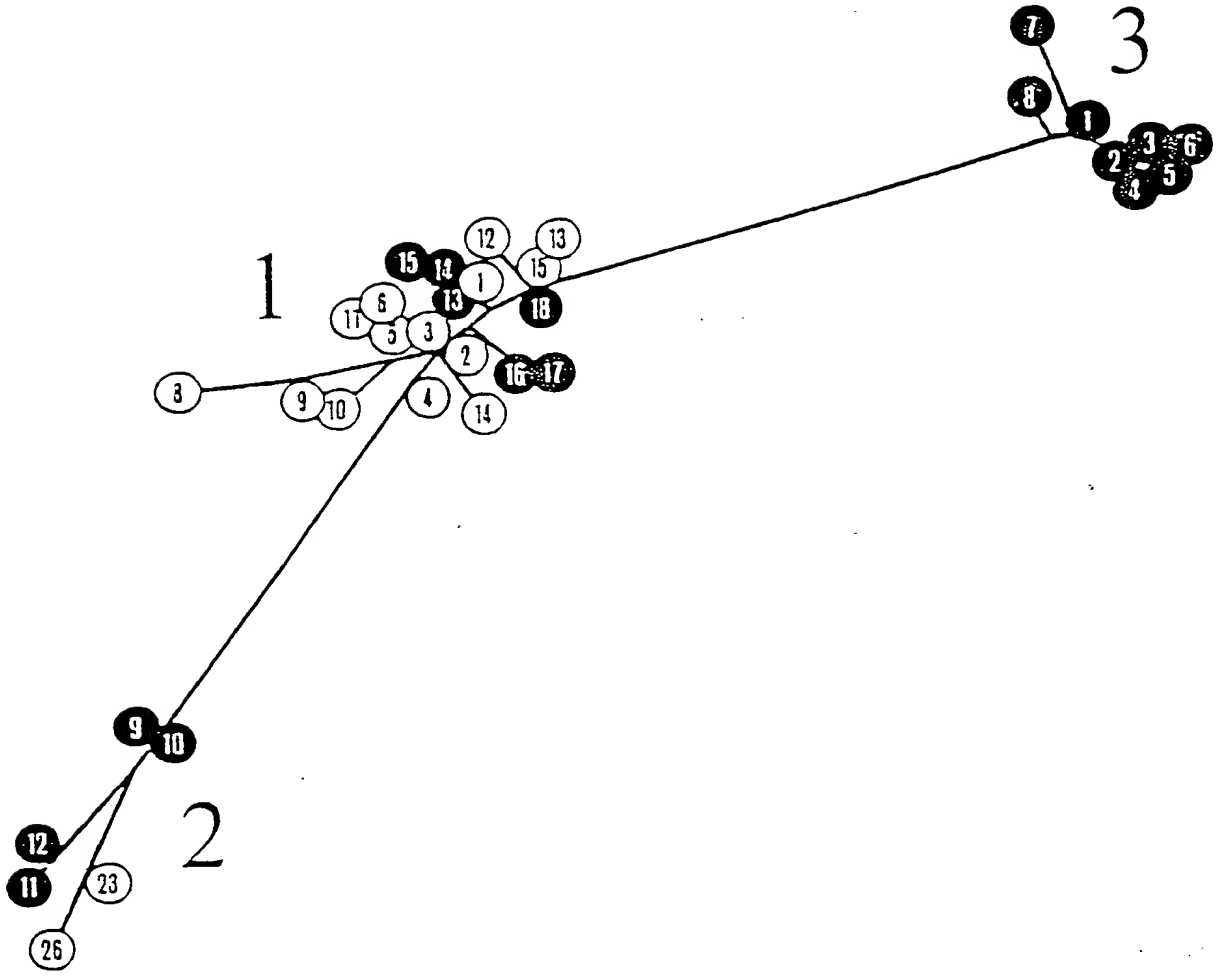

1
2
3
7
8
1
2
3
4
5
6
12
13
15
14
1
13
15
18
11
6
5
3
2
16
17
8
9
10
4
14
9
10
12
11
23
26

FIGURE 3

		2648	2668	2688	2708	2732			
	E-b1	VTEQDIRVEE	EIYQCCNLEP	EARKVISSLT	ERLYCGGPMF	NSKGAQCGYR	RCRASGVLPT	SFGNTITCYI	KATAACEAAG	LRNPD
	E-b2	....	....	....	....	....	....	....	.....K....	.....
	E-b3	....	....	....	....	....	....	....	.....K....	.....
	E-b7	....	....	....	....	....	....	....	.....K....	.....
2	K2a	...R...T..	S..RA.S.PE	..HIA.H...	....V.....	....QT....	........T.	.M.......V	..L...K...	IVA.S
2	K2a-1	...R...T..	S...A.S.PE	...TA.H...	....V.....	....QT....	........T.	.M.......V	..L...K...	IVA.T
	E-b12	..	S...A.S.PQ	...T..H...	....V....T	....QS....	.......FT.	.M...M....	..L...K...	IVD.V
2	K2b	...R...T..	S...A.S.PQ	...TA.H...	....V....T	....QS....	.......FT.	.M...M....	..L...K...	IVD.I
2	K2b-1	...R...T..	S...A.S.PE	...T..H...	....V....T	....QS....	.......FT.	.M...M....	..L...K...	IVD.I
1	HCV-1	...S...T..	A.....D.D.	Q..VA.K...	....V...LT	..R.EN....	........T.	.C...L....	..R...R...	.QDCT
1	Pt-1	...S...T..	A.....D.D.	Q..VA.K...	....V...LT	..R.EN....	........T.	.C...L....	..R...R...	..DCT
1	H77	...S...T..	A.....D.D.	Q..VA.K...	....V...LT	..R.EN....	........T.	.C...L....	..R...R...	.QDCT
1	H90	...S...T..	A.....D.D.	Q..TA.K...	....V...LT	..R.EN....	........T.	.C...L....	..R...R...	.QDCT
1	HCV-J	...N...T..	S.....D.A.	...QA.R...	....V...LT	....QN....	........T.	.C...L...L	......R..K	.QDCT
1	HCV-BK	...N......	S.....D.A.	...QA.K...	....I...LT	....QN....	........T.	.C...L...L	..S...R..K	.QDCT
1	K1	...N......	S...S.D.A.	...QA.R...	....I...LT	....QN....	........T.	.C...L...L	......R..K	.QDCT
1	K1-1	...N......	S.....A.A.	...QA.R...	....I...LT	....QN....	........T.	.C...L...L	..S...R..K	.QDCT
1	K1-2	...S......	S.....D.A.	...Q..R...	....I...LT	....QN....	........T.	.C...L...L	..S...R..K	..DCT
1	K1-3	...N......	S.....D.A.	..KLA.R...	....I...LT	....QN....	........T.	.C...L...L	..S...R..K	.QDCT
1	K1-4	...N......	S.....D.A.	...Q..R...	....I...LT	....QN....	........T.	.C...L...L	..S...R..K	.QDCT

FIGURE 4

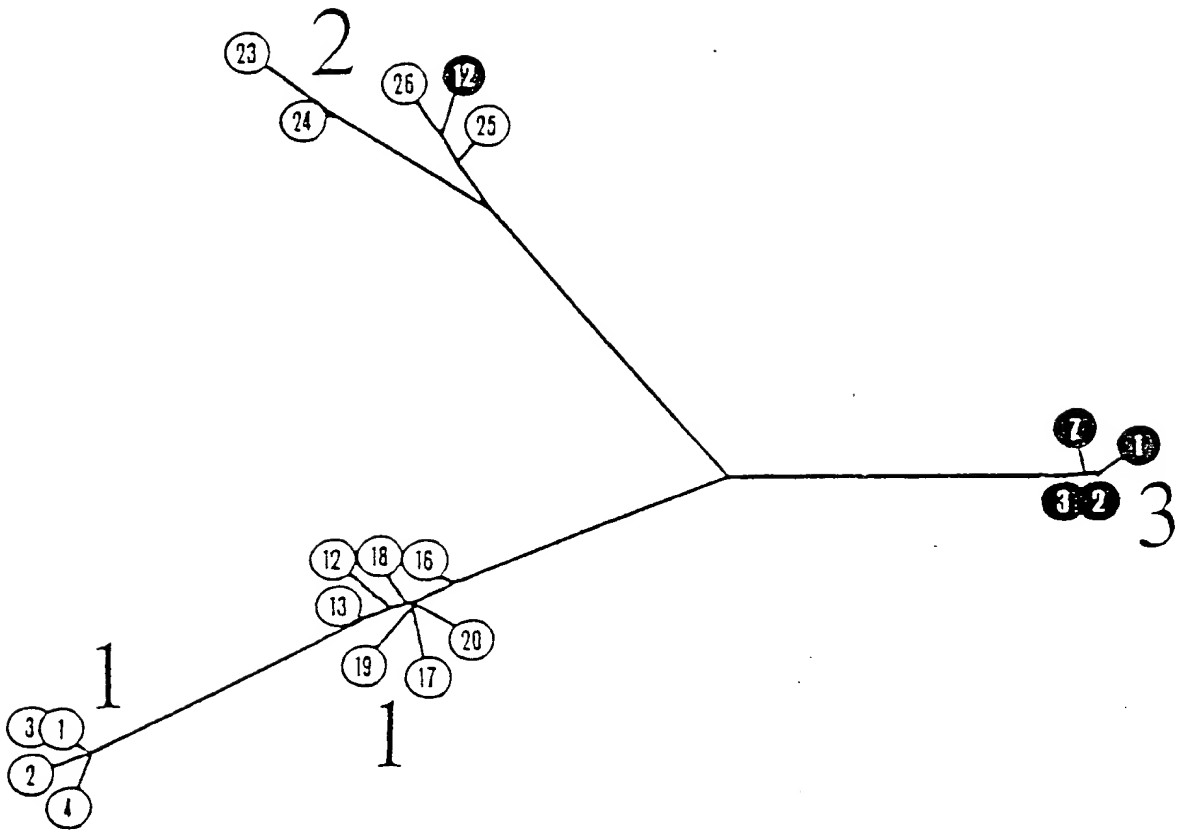


FIGURE 5

		1577		1597		1617		1633
	E-b1	KQQGLNFSYL	TAYQATVCAR	AQALPPSWDE	TWKCLVRLKP	TLHGPTPLLY		RLGPVQN
	E-b2	....	....	...P.....	....	....		.....
	E-b6	....	....	...P.....	M.........	....		.....
	E-b7	....	A.........	...P.....	....	....		.....
?	Clone A	..G.D..A..	....	.K.P.....V	M....A....	..I.....		...A.T.
	E-b16	..S.E.LP..	V.........	...P.....Q	M....I....	....		...A...
	E-b17	..S.E.LP..	V.........	...P.....Q	M....I....	....		...A...
	Group 1/1	..S.E.LP..	V.........	...P.....Q	M....I....	....		...A...
	1/2	..S.E.LP..	V.........	...P.....Q	M..R.I....	....		.
	1/3	..S.E..P..	V.........	...P.....Q	M....I....	....		..
	1/4	..S.E..P..	V.........	...P.....Q	M....I...A	....		..
	1/4	..S.E..P..	V.........	...P....EQ	M....I...A	....		...A.
1	HCV-1	..S.E.LP..	V.........	...P.....Q	M....I....	....		...A...
1	H77	..S.E..P..	V.........	...P.....Q	M....I....	....		...A...
1	H90	..S.E..P..	V.........	...P.....Q	M....I....	....		...A...
1	HCV-J	..A.D.LP..	V.........	...P.....Q	M....I....	....		...A...
1	HCV-BK	..A.D..P..	V.........	...P.....Q	M....I....	....		...A...
1	JH	..A.D..P..	V.........	.K.P.....Q	M....I....	....		...A...

FIGURE 6

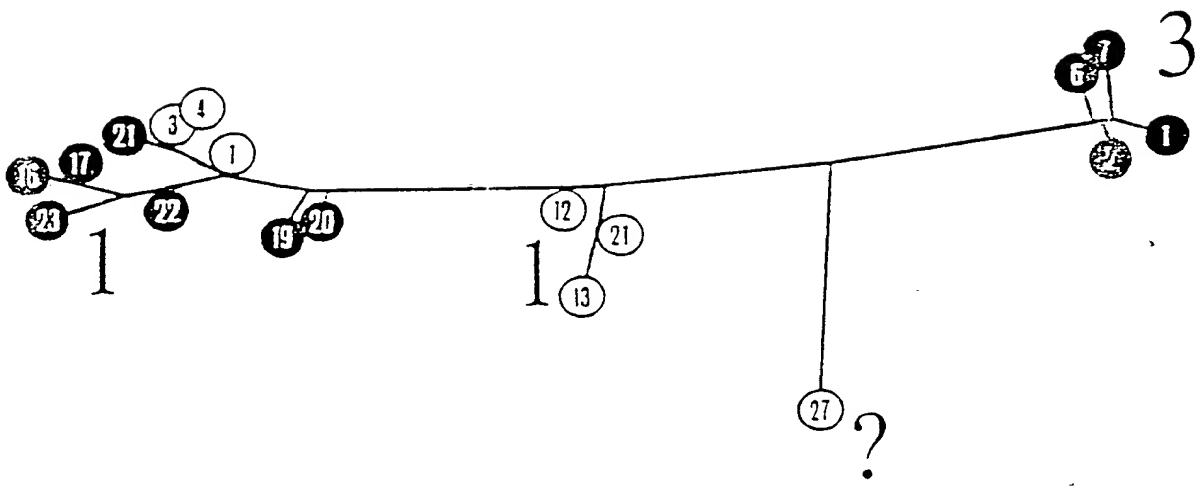


FIGURE 7

		5		25		45	64
	E-b1	AKPQRKTKRN	TIRRPQDVKF	PGGGQIVGGV	YVLPRRGPRL	GVCATRKTSE	RSQPRGRRQP
1	HCV-1	P...K.N...	.N.....	.....	.L.....	..R.....	.....
1	H77	P.....	.N.....	.....	.L.....	..R.....	.....
1	H90	P.....	.N.....	.....	.L.....	..R.....	.....
1	GM1	.....	.N.....	.....	.L.....	..R.P....	.....
1	GM2	.....	.N.....	.....	.L.....	..R.....	.....
1	HCV-J	P.....	.N.....	.....	.L.....	..R.....	.....
1	HCV-BK	P.....	.N.....	.....	.L.....	..R.P....	.....
1	HC-J1	P.....	.N.....	.....	.L.....	..R.....	.....
1	HC-J4	P.....	.N.....	.....	.L.....	..R.....	.....W...
1	JH	P.....	.N.....	.....	.L.....	..R.....	.....
1	J7	P.....	.N.....	.....	.L.....	..R.....	.....

		65	85		105		128
	E-b1	IPKARRSEGR	SWAQPGYPWP	LYGNEGCGWA	GWLLSPRGSR	PSWGPNDPRR	RSRNLGKVID	TLTW
1	HCV-1	.....P...	T.....	.....	.....	.....T....	.....	...C
1	H77	.....P...	T.....	.....	.....	.....T....	.....	...C
1	H90	.....P...	T.....	.....M	.....	.....T....	.....	.F.C
1	GM1	.....P...	T.....	.....	.....	.....T....	.....	...C
1	GM2	.....L...	T.....	.....	.....	.....T....	.....	...C
1	HCV-J	.....P...	T.....	.....M...	.....	.....T....	.....	...C
1	HCV-BK	.....P...	T.....	.....L...	.....	.....T....	.....	...C
1	HC-J1	...V..P...	T.....	.....	.....	.....T....	.....	...C
1	HC-J4	.....P...	A.....	.....L...	.....	.....T....	.....	...C
1	JH	.....P...	T.....	.....L...	.....	.....	.....	...C
1	J7	.....P...	T.....	.....L...	.....	.....	.....	...C

FIGURE 8

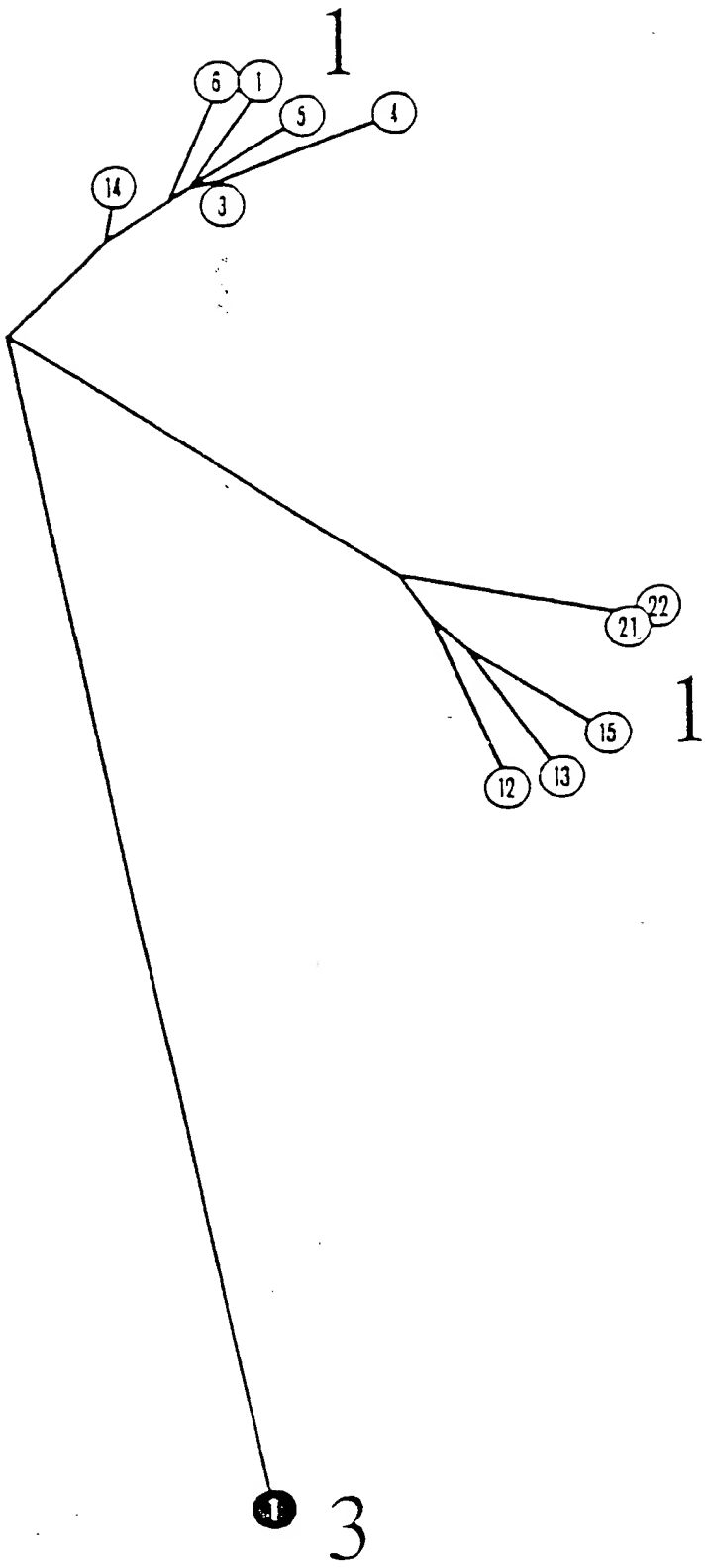


FIGURE 9a

NUCLEOTIDE SEQUENCES

```
    4911                                                                4970
T0040 GACACACCCU GUCACAAAAU ACAUCAUGGC AUGCAUGUCA GCUGAUCUGG AAGUAACCAC
T0038 ??CACACCCC GUCACAAAAU ACAUCAUGGC AUGCAUGUCA GCUGAUCUGG AAGUAACCAC
T0036 ??CACACCCC AUCACAAAAU ACAUCAUGGC AUGCAUGUCA GCUGAUCUGG AAGUAACCAC
T0026 ????CACCCC AUCGCGAAAU ACGUCAUGGC AUGUAUGUCA GCUGAUCUGG AAGUAACCAC
T1787 ????CACCCC AUCACAAAAU ACGUCAUGGC AUGCAUGUCA GCUGAUCUGG AAGUAACCAC
      ---------- ---------- ---------- ---------- ---------- ----------
Cons  GACACACCCU GUCACAAAAU ACAUCAUGGC AUGCAUGUCA GCUGAUCUGG AAGUAACCAC

    4971                                                                5030
T0040 CAGCACCUGG GUGUUGCUUG GAGGGGUCCU CGCGGCCCUA GCGGCCUACU GCUUGUCAGU
T0038 CAGCACCUGG GUGUUGCUUG GAGGGGUCCU CGCGGCCCUA GCGGCCUACU GCUUGUCAGU
T0036 CAGCACCUGG GUGUUGCUUG GAGGGGUCCU CGCGGCCCUA GCGGCCUACU GCUUGUCAGU
T0026 CAGCACCUGG GUGUUGCUUG GAGGAGUCCU CGCUGCCCUA GCGGCCUACU GCUUGUCAGU
T1787 CAGCACCUGG GUGUUGCUUG GAGGGGUCCU CGCGGCCCUA GCGGCCUACU GCUUGUCAGU
      ---------- ---------- ---------- ---------- ---------- ----------
Cons  CAGCACCUGG GUGUUGCUUG GAGGGGUCCU CGCGGCCCUA GCGGCCUACU GCUUGUCAGU

    5031                                                                5090
T0040 CGGCUGCGUU GUGAUUGUGG GUCAUAUUGA GCUGGGGGGC AAGCCGGCA AUCGUUCCAGA
T0038 CGGCUGCGUU GUGAUUGUGG GCCAUAUUGA GCUGGGGGGC AAGCCGGCA CUCGUUCCAGA
T0036 CGGCUGCGUU GUGAUUGUGG GCCAUAUUGA GCUGGGGGGC AAGCCGGCA CUCGUUCCAGA
T0026 CGGCUGCGUU GUGAUUGUGG GUCAUAUUGA GCUGGGGGGC AAGCCAGCA CUCGUUCCAGA
T1787 CGGCUGCGUU GUGAUUGUGG GUCAUAUUGA GCUGGGAGGC AAGCCGGCA CUCGUUCCAGA
      ---------- ---------- ---------- ---------- ---------- ----------
Cons  CGGCUGCGUU GUGAUUGUGG GUCAUAUUGA GCUGGGGGGC AAGCCGGCA AUCGUUCCAGA

    5091                                                                5150
T0040 CAAAGAGGUG UUGUAUCAAC AAUACGAUGA GAUGGAGGAG UGCUCGCAA GCUGCCCCAUA
T0038 CAAAGAAGUG UUGUAUCAAC AAUACGAUGA GAUGGAGGAG UGCUCGCAA GCCGCCCCAUA
T0036 CAAAGAGGUG UUGUAUCAAC AAUACGAUGA GAUGGAGGAG UGCUCGCAA GCCGCCCCAUA
T0026 CAAAGAGGUG UUGUAUCAAC AAUACGAUGA GAUGGAGGAG UGCUCGCAA GCCGCCCCAUA
T1787 CAAGGAGGUG UUGUAUCAAC AAUACGAUGA GAUGGAGGAG UGCUCGCAA GCCGCCCCAUA
      ---------- ---------- ---------- ---------- ---------- ----------
Cons  CAAAGAGGUG UUGUAUCAAC AAUACGAUGA GAUGGAGGAG UGCUCGCAA GCUGCCCCAUA

    5151                                                                5210
T0040 UAUCGAACAA GCUCAGGUGA UAGCCCACCA GUUCAAGGAG AAAGUCCUU GGAUUGCUGCA
T0038 UAUCGAACAA GCUCAAGUAA UAGCCCACCA GUUCAAGGAG AAAGUCCUU GGAUUGCUGCA
T0036 UAUCGAACAA GCUCAGGUAA UAGCCCACCA GUUCAAGGAG AAAGUCCUU GGAUUGCUGCA
T0026 UAUCGAACAA GCUCAGGUAA UAGCCCACCA GUUCAAGGAG AAAGUCCUU GGAUUGCUGCA
T1787 UAUCGAACAA GCUCAGGUAA UAGCCCACCA GUUCAAGGAG AAAGUCCUU GGGUUGCUGCA
      ---------- ---------- ---------- ---------- ---------- ----------
Cons  UAUCGAACAA GCUCAGGUGA UAGCCCACCA GUUCAAGGAG AAAGUCCUU GGAUUGCUGCA

    5211                                                                5270
T0040 GCGAGCCACC CAACAACAAG CUGUUAUUGA GCCAAUAGUA GCUACCAAC UGGCAAAAGCU
T0038 GCGGGCCACC CAACAACAAG CUGUUAUUGA GCCCAUAGUA GCUACCAAC UGGCAAAAGCU
T0036 GCGAGCCACC CAACAACAAG CUGUUAUUGA GCCAAUAGUA GCUACCAAC UGGCAAAA???
T0026 GCGAGCCACC CAACAACAAG CUGUCAUUGA GCCCAUAGUA GCUACCAAC UGGCAAAA???
T1787 GCGAGCCACC CAACAACAGG CUGUCAUUGA GCCCAUAGUA GCUACCAAC UGGCAAAAGCU
      ---------- ---------- ---------- ---------- ---------- ----------
Cons  GCGAGCCACC CAACAACAAG CUGUUAUUGA GCCCAUAGUA GCUACCAAC UGGCAAAAGCU

    5271
T0040 UGAGACC
T0038 UGAGGCC
T0036 ???????
T0026 ???????
T1787 UGAGGCU
      -------
Cons  UGAGACC
```

FIGURE 9b

DEDUCED AMINO ACID SEQUENCES

```
                                                                    1787
    1638                                                             ▼
      ▼
T0040 THPVT KYIMA CMSAD LEVTT STWVL LGGVL AALAA YCLSV GCVVI VGHIE
T0038 .HPVT KYIMA CMSAD LEVTT STWVL LGGVL AALAA YCLSV GCVVI VGHIE
T0036 .HPIT KYIMA CMSAD LEVTT STWVL LGGVL AALAA YCLSV GCVVI VGHIE
T0026 .HPIA KYLMA CMSAD LEVTT STWVL LGGVL AALAA YCLSV GCVVI VGHIE
T1787 .HPIT KYVMA CMSAD LEVTT STWVL LGGVL AALAA YCLSV GCVVI VGHIE
      ----- ----- ----- ----- ----- ----- ----- ----- ----- -----
Cons  THPVT KYIMA CMSAD LEVTT STWVL LGGVL AALAA YCLSV GCVVI VGHIE

                                                                    1737
    1688                                                             ▼
      ▼
T0040 LGGKP AIVPD KEVLY QQYDE MEECS QAAPY IEQAQ VIAHQ FKEKV LGLLQ
T0038 LGGKP ALVPD KEVLY QQYDE MEECS QAAPY IEQAQ VIAHQ FKEKV LGLLQ
T0036 LGGKP ALVPD KEVLY QQYDE MEECS QAAPY IEQAQ VIAHQ FKEKV LGLLQ
T0026 LGGKP ALVPD KEVLY QQYDE MEECS QAAPY IEQAQ VIAHQ FKEKV LGLLQ
T1787 LGGKP ALVPD KEVLY QQYDE MEECS QAAPY IEQAQ VIAHQ FKEKV LGLLQ
      ----- ----- ----- ----- ----- ----- ----- ----- ----- -----
Cons  LGGKP ALVPD KEVLY QQYDE MEECS QAAPY IEQAQ VIAHQ FKEKV LGLLQ

    1738                                                  1765
      ▼                                                    ▼
T0040 RATQQ QAVIE PIVAT NWQKL ETFWH KHM
T0038 RATQQ QAVIE PIVAT NWQKL EAFWH KHM
T0036 RATQQ QAVIE PIVAT NWQ.. ..... ...
T0026 RATQQ QAVIE PIVAT NWQ.. ..... ...
T1787 RATQQ QAVIE PIVAT NWQKL EAFWH KHM
      ----- ----- ----- ----- ----- ---
Cons  RATQQ QAVIE PIVAT NWQKL EAFWH KHM
```

FIGURE 10a

SEQUENCE COMPARISONS WITH TYPE 3

```
                                        TYPE 3
T0040  CVVIVGHIELGGKPAIVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNWQKLETFWHKHM...
T0038  CVVIVGHIELGGKPALVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNWQKLEAFWHKHM...
T0036  CVVIVGHIELGGKPALVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNW.............
T0026  CVVIVGHIELGGKPALVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNWQ............
T1787  CVVIVGHIELGGKPALVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNWQKLEAFWHKHM...

                                        TYPE 2
T0351  ...II..L.INQRAVIAPDKEVLYEAFDEMEECASRAALIEEGQRIAEMLRSKIQGLLQQASKQAQDIQPAVQASWPKVEQFW.......
T0059  CISIIGRLHLNDRVVVTPDKEILYEAFDEMEECASKAALIEEGQRMAEMLKSKIQGLLQQATRQAQDIQPVVQSSWPKLEQFWAKHM...
T0940  CVSIIGRLHL..RVV...DK...YE..........AALIEEGQRMAEMLKSKIQGLLQQATRQAQDIQPVVQSSWPKLEQFW.......
T0810  CISIIGRLHLNDRVVVAPDKEILYEAFDEMEECASKAALIEEGQRMAEMLKSKIQGLLQQATRQAQDIQPAVQSSWPKLEQFW.......

                                        TYPE 1
T0016  CVVIVGRIVLSGKPAIIPDRE.............PYIEQGMMLAEQFKQKALGLLQTASRQAEVIAP..QTNWQRLETF........
T0042  CVVIVGRIVLSGKPAIIPDREVLYREFDEMEECSQHLPYIEQGMMLAEQFKQKALGLLQTASRQAEVIAPAVQTNWQRLEAF........
T0077  CVVIVGRIVLSGKPAIIPDREVLYREFDEMEECSQHLPYIEQGMMLAEQFKQKALGLLQTASRQAEVIAPAVQTNWQRLEAFW.......
T1801  CVVIVGRIVLSGKPAVIPDREVLYREFDEMEECSQHLPYIEQGMALAEQFKQKALGLLQTASRQAEVIAPAVQTNWQKLEAFWAK.....
T1825  CVVIVGRIDLSGKPAVIPDREVLYREFDEMEECSQHLPYIEQGMALAEQFKQKALGLLQTASRQAEVITPVVQTNWQKLEAFWAKHM...
```

FIGURE 10b

DERIVATION OF PEPTIDE SEQUENCES

TYPE 3

```
T0040  CVVIVGHIELGGKPAIVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNWQKLETFWHKHM
T0038  CVVIVGHIELGGKPALVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNWQKLEAFWHKHM
T0036  CVVIVGHIELGGKPALVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNW..........
T0026  CVVIVGHIELGGKPALVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNWQ.........
T1787  CVVIVGHIELGGKPALVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNWQKLEAFWHKHM
-------------------------------------------------------------------------------------------
Peptd  CVVIVGHIELGGKPALVPDKEVLYQQYDEMEECSQAAPYIEQAQVIAHQFKEKVLGLLQRATQQQAVIEPIVATNWQKLEAFWHKHM
```

TYPE 2

```
T0351  ...II..L.INQRAVIAPDKEVLYEAFDEMEECASRAALIEEGQRIAEMLRSKIQGLLQQASKQAQDIQPAVQASWPKVEQFW.......
T0059  CISIIGRLHLNDRVVVTPDKEILYEAFDEMEECASKAALIEEGQRMAEMLKSKIQGLLQQATRQAQDIQPVVQSSWPKLEQFWAKHMW
T0940  CVSIIGRLHL..RVV...DK...YE..........AALIEEGQRMAEMLKSKIQGLLQQATRQAQDIQPVVQSSWPKLEQFW.....
T0810  CISIIGRLHLNDRVVVAPDKEILYEAFDEMEECASKAALIEEGQRMAEMLKSKIQGLLQQATRQAQDIQPAVQSSWPKLEQFW.....
-------------------------------------------------------------------------------------------
Peptd  CISIIGRLHLNDRVVVTPDKEILYEAFDEMEECASKAALIEEGQRMAEMLKSKIQGLLQQATRQAQDIQPVVQSSWPKLEQFWAKHMW
```

TYPE 1

```
T0016  CVVIVGRIVLSGKPAIIPDRE..............PYIEQGMMLAEQFKQKALGLLQTASRQAEVIAP..QTNWQRLETF........
T0042  CVVIVGRIVLSGKPAIIPDREVLYREFDEMEECSQHLPYIEQGMMLAEQFKQKALGLLQTASRQAEVIAPAVQTNWQRLEAF........
T0077  CVVIVGRIVLSGKPAIIPDREVLYREFDEMEECSQHLPYIEQGMMLAEQFKQKALGLLQTASRQAEVIAPAVQTNWQRLEAFW.......
T1801  CVVIVGRIVLSGKPAVIPDREVLYREFDEMEECSQHLPYIEQGMALAEQFKQKALGLLQTASRQAEVIAPAVQTNWQKLEAFWAK.....
T1825  CVVIVGRIDLSGKPAVIPDREVLYREFDEMEECSQHLPYIEQGMALAEQFKQKALGLLQTASRQAEVITPVVQTNWQKLEAFWAKHM...
-------------------------------------------------------------------------------------------
Peptd  CVVIVGRIVLSGKPAIIPDREVLYREFDEMEECSQHLPYIEQGMMLAEQFKQKALGLLQTASRQAEVIAPAVQTNWQRLEAFWAKHMWHF
```

FIGURE 11a

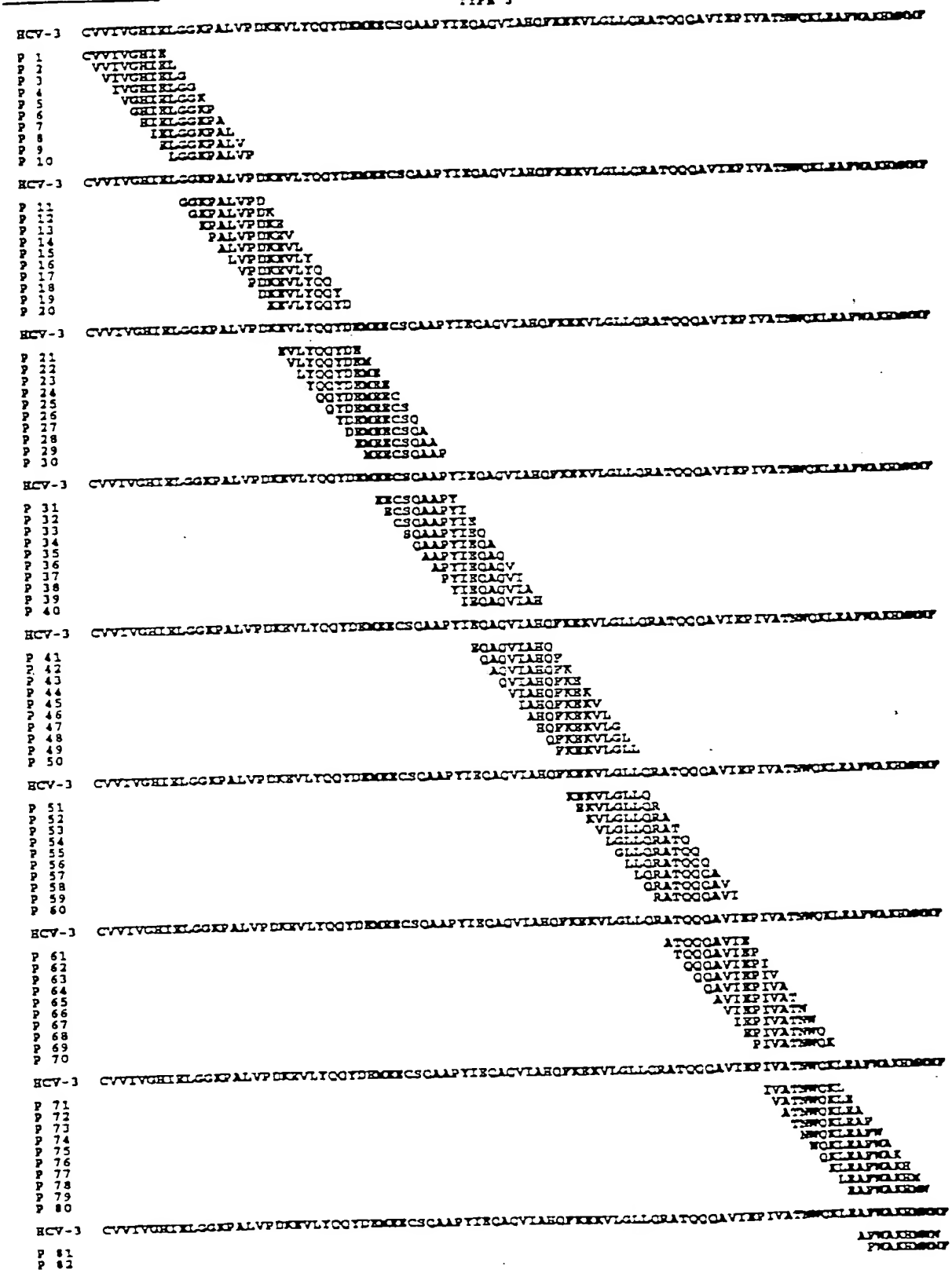

TYPE 3
HCV-3
P 1
P 2
P 3
P 4
P 5
P 6
P 7
P 8
P 9
P 10
P 11
P 12
P 13
P 14
P 15
P 16
P 17
P 18
P 19
P 20
P 21
P 22
P 23
P 24
P 25
P 26
P 27
P 28
P 29
P 30
P 31
P 32
P 33
P 34
P 35
P 36
P 37
P 38
P 39
P 40
P 41
P 42
P 43
P 44
P 45
P 46
P 47
P 48
P 49
P 50
P 51
P 52
P 53
P 54
P 55
P 56
P 57
P 58
P 59
P 60
P 61
P 62
P 63
P 64
P 65
P 66
P 67
P 68
P 69
P 70
P 71
P 72
P 73
P 74
P 75
P 76
P 77
P 78
P 79
P 80
P 81
P 82

FIGURE 11b

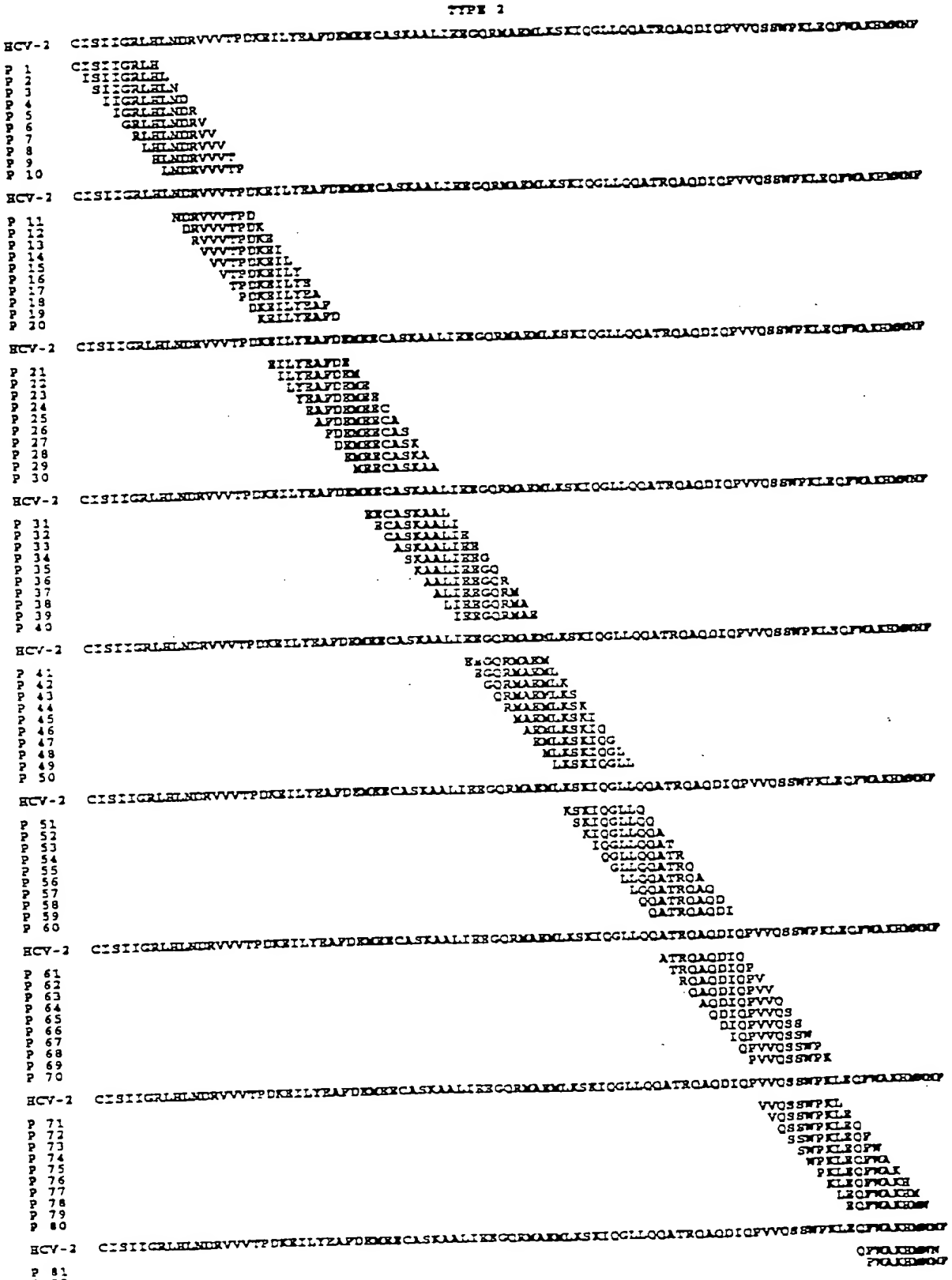
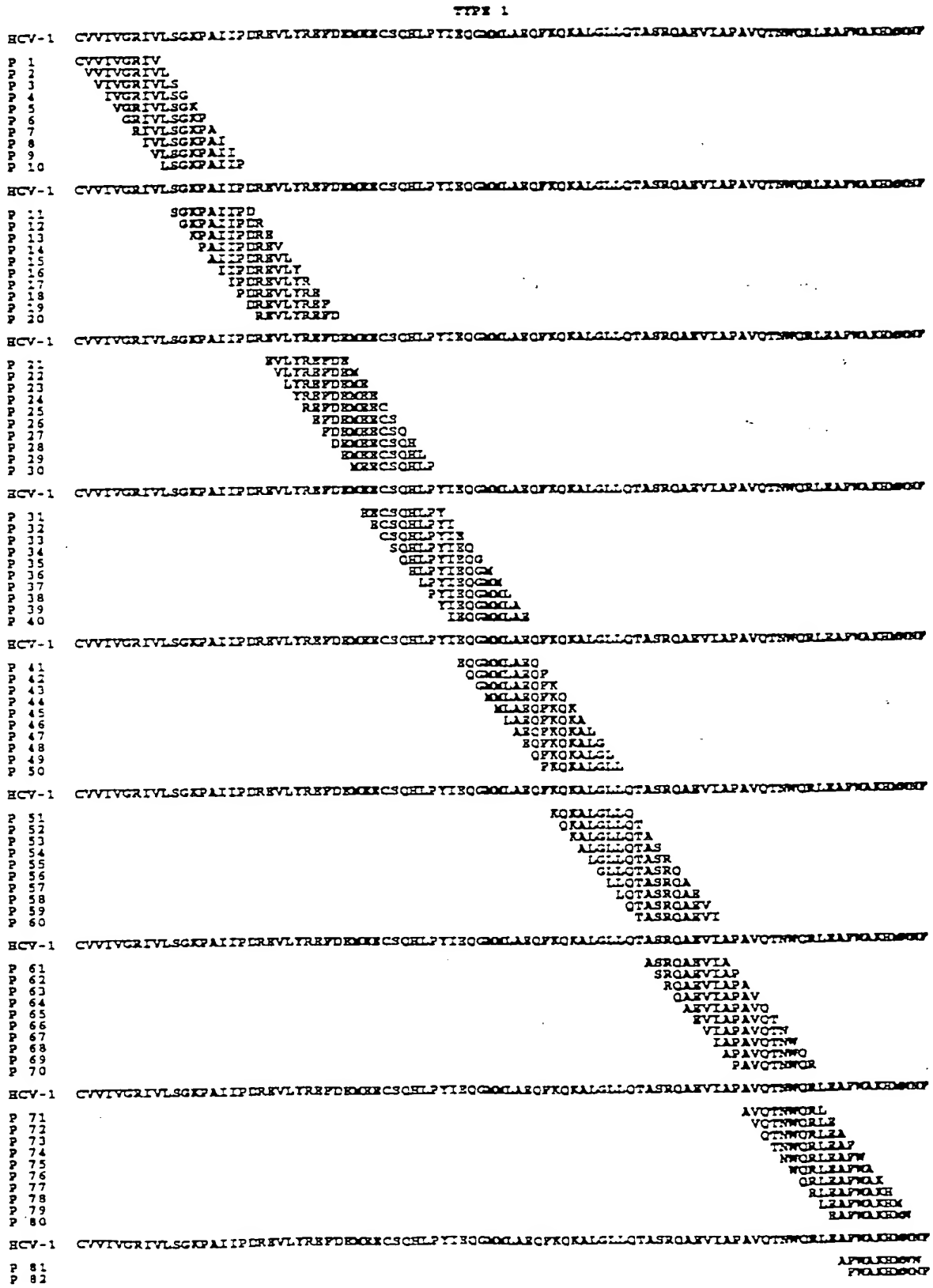

TYPE 2
HCV-2
P 1
P 2
P 3
P 4
P 5
P 6
P 7
P 8
P 9
P 10
P 11
P 12
P 13
P 14
P 15
P 16
P 17
P 18
P 19
P 20
P 21
P 22
P 23
P 24
P 25
P 26
P 27
P 28
P 29
P 30
P 31
P 32
P 33
P 34
P 35
P 36
P 37
P 38
P 39
P 40
P 41
P 42
P 43
P 44
P 45
P 46
P 47
P 48
P 49
P 50
P 51
P 52
P 53
P 54
P 55
P 56
P 57
P 58
P 59
P 60
P 61
P 62
P 63
P 64
P 65
P 66
P 67
P 68
P 69
P 70
P 71
P 72
P 73
P 74
P 75
P 76
P 77
P 78
P 79
P 80
P 81
P 82

FIGURE 11c


TYPE 1
HCV-1
P 1
P 2
P 3
P 4
P 5
P 6
P 7
P 8
P 9
P 10
P 11
P 12
P 13
P 14
P 15
P 16
P 17
P 18
P 19
P 20
P 21
P 22
P 23
P 24
P 25
P 26
P 27
P 28
P 29
P 30
P 31
P 32
P 33
P 34
P 35
P 36
P 37
P 38
P 39
P 40
P 41
P 42
P 43
P 44
P 45
P 46
P 47
P 48
P 49
P 50
P 51
P 52
P 53
P 54
P 55
P 56
P 57
P 58
P 59
P 60
P 61
P 62
P 63
P 64
P 65
P 66
P 67
P 68
P 69
P 70
P 71
P 72
P 73
P 74
P 75
P 76
P 77
P 78
P 79
P 80
P 81
P 82

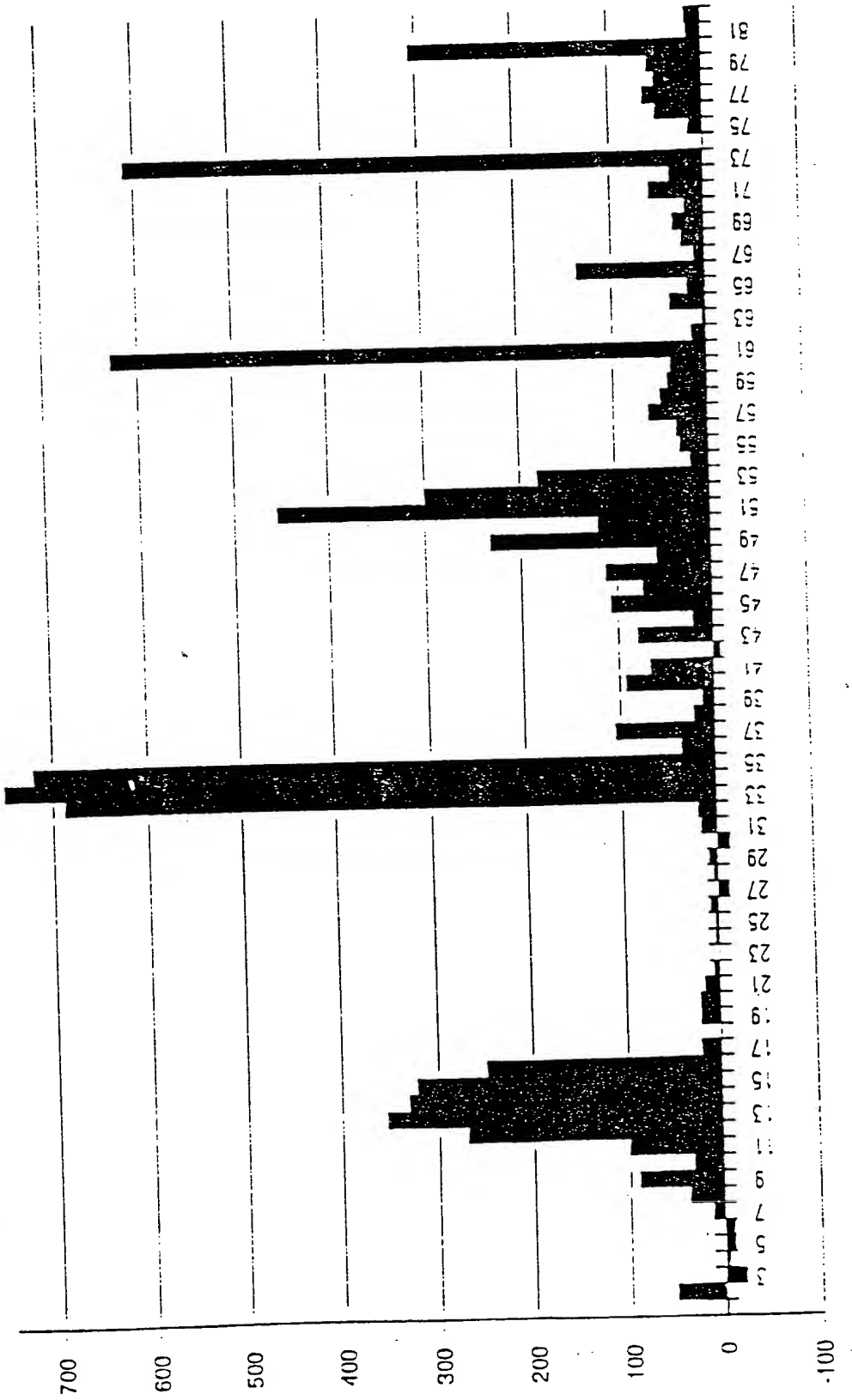

700
600
500
400
300
200
100
0
-100
1
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
41
43
45
47
49
51
53
55
57
59
61
63
65
67
69
71
73
75
77
79
81

FIGURE 12a

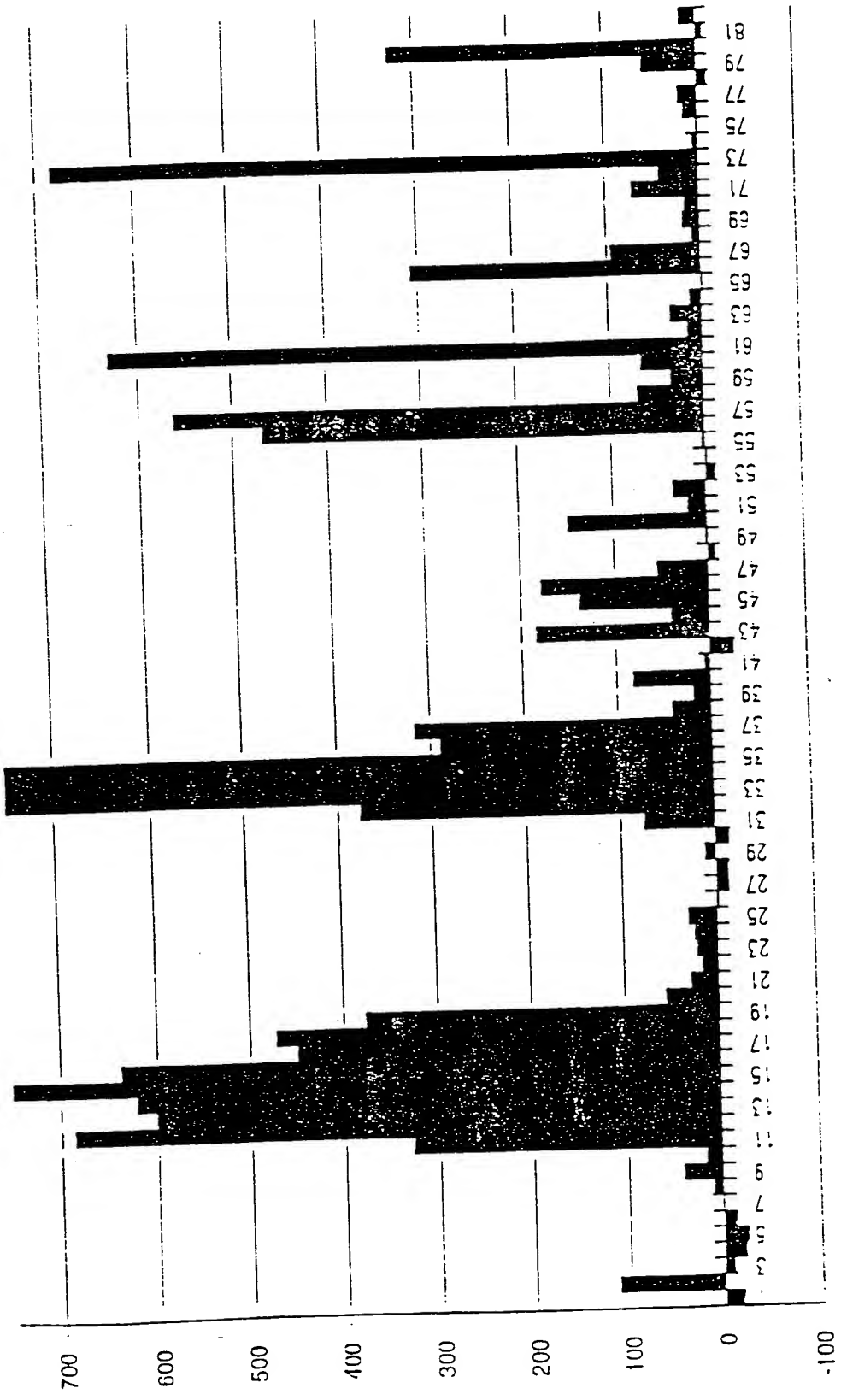

700
600
500
400
300
200
100
0
-100
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
41
43
45
47
49
51
53
55
57
59
61
63
65
67
69
71
73
75
77
79
81

FIGURE 12b

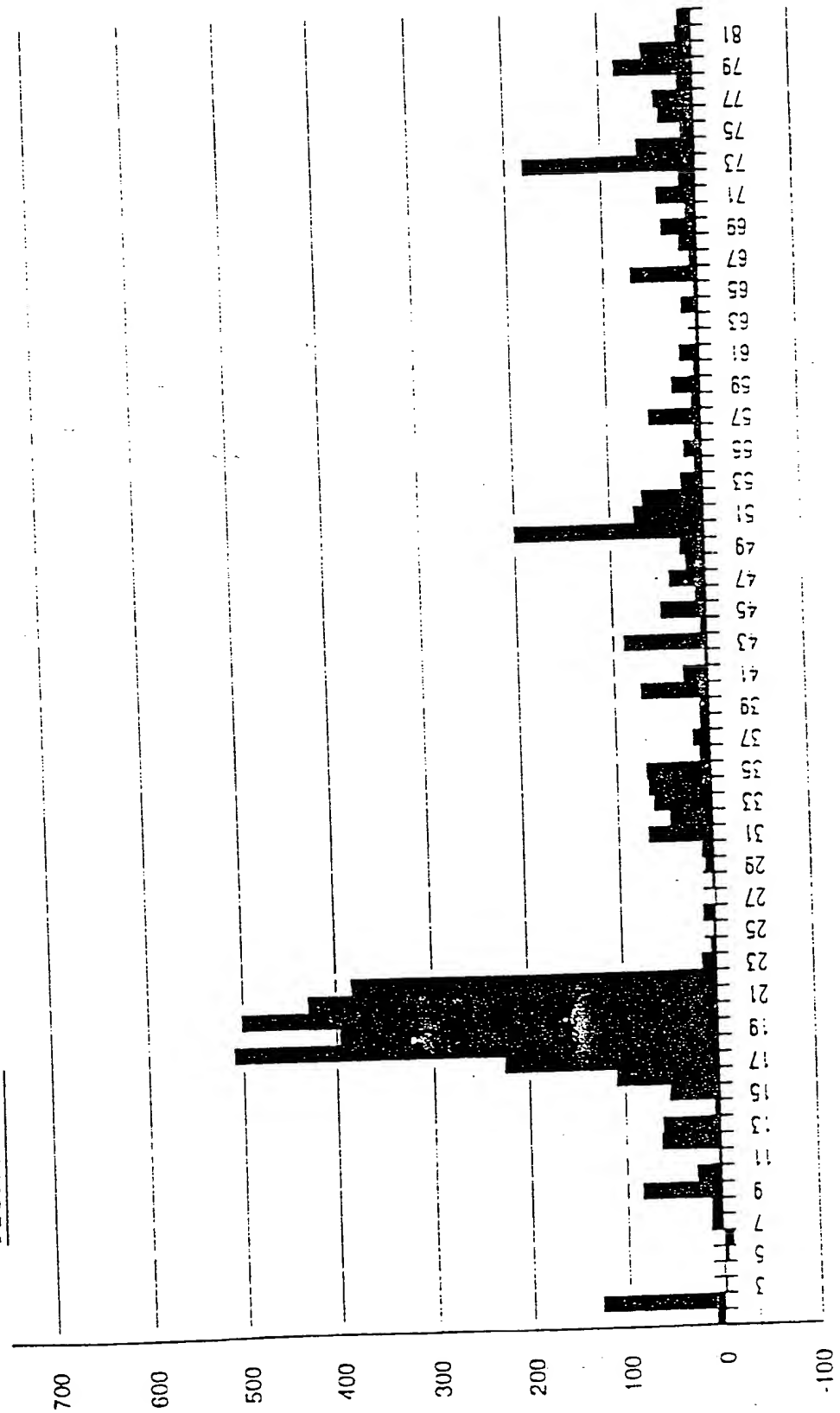
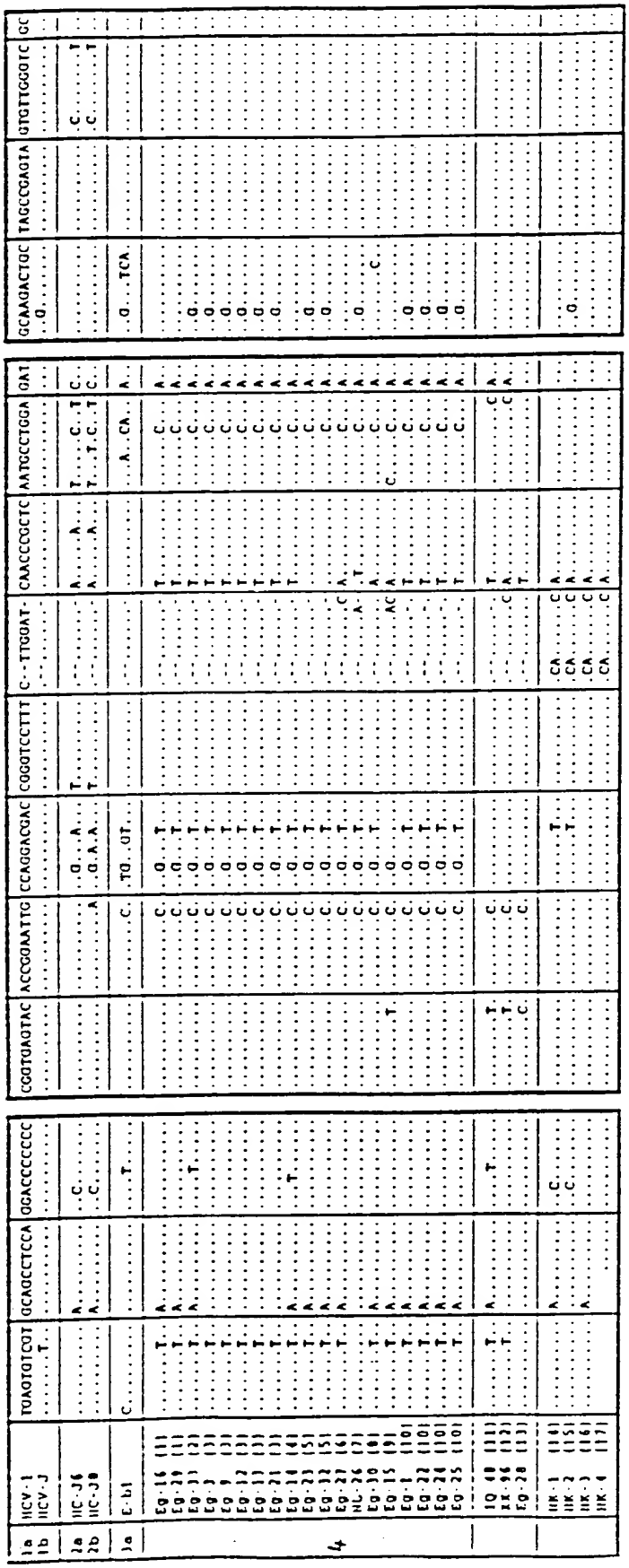

700
600
500
400
300
200
100
0
-100
3
5
7
9
11
13
15
17
19
21
23
25
27
29
31
33
35
37
39
41
43
45
47
49
51
53
55
57
59
61
63
65
67
69
71
73
75
77
79
81

FIGURE 12c

FIGURE 13



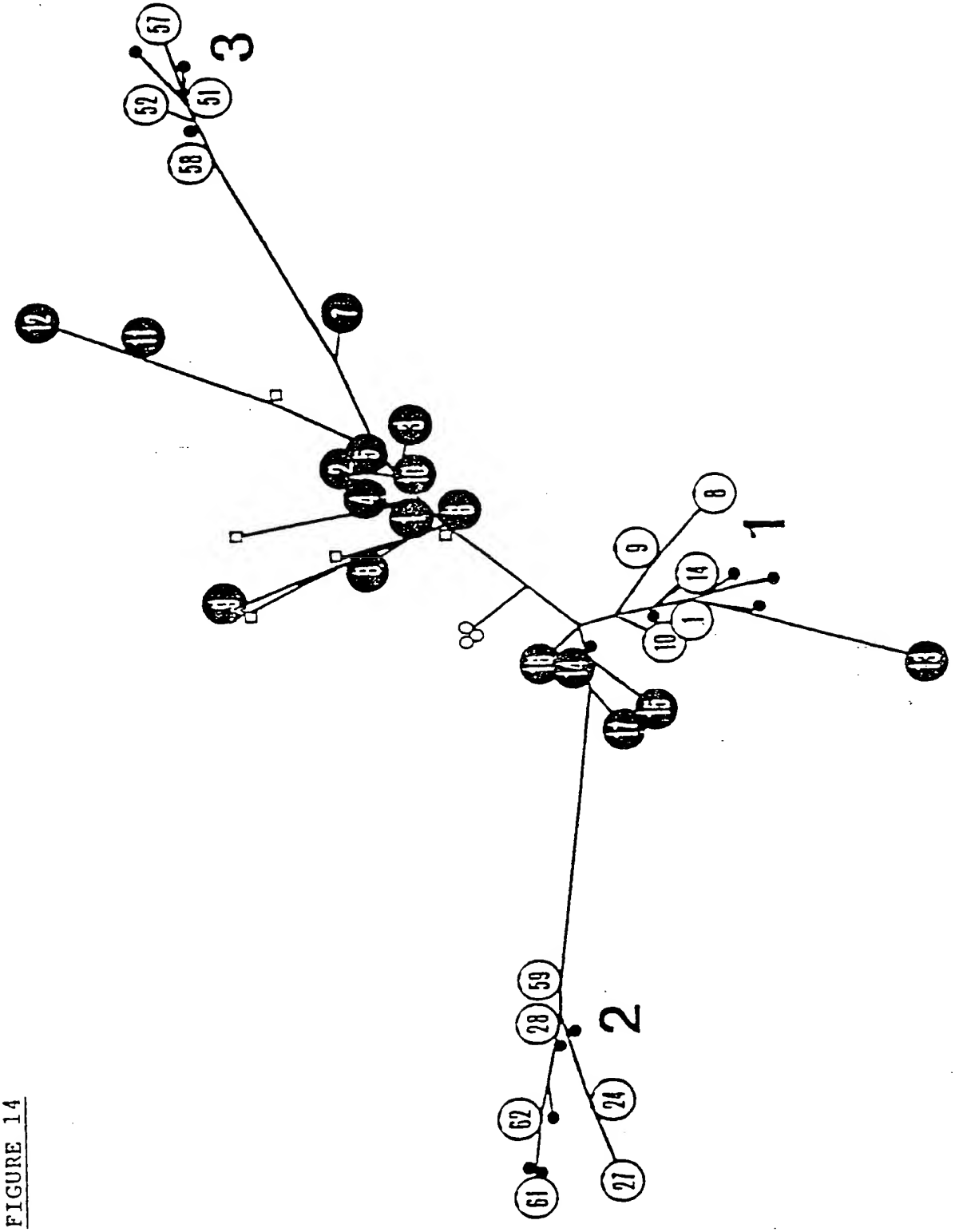

3
1
2
57
52
51
58
12
11
7
3
2
6
10
4
5
8
9
8
9
14
1
10
13
15
11
59
28
24
62
27
61

FIGURE 14

FIGURE 15a

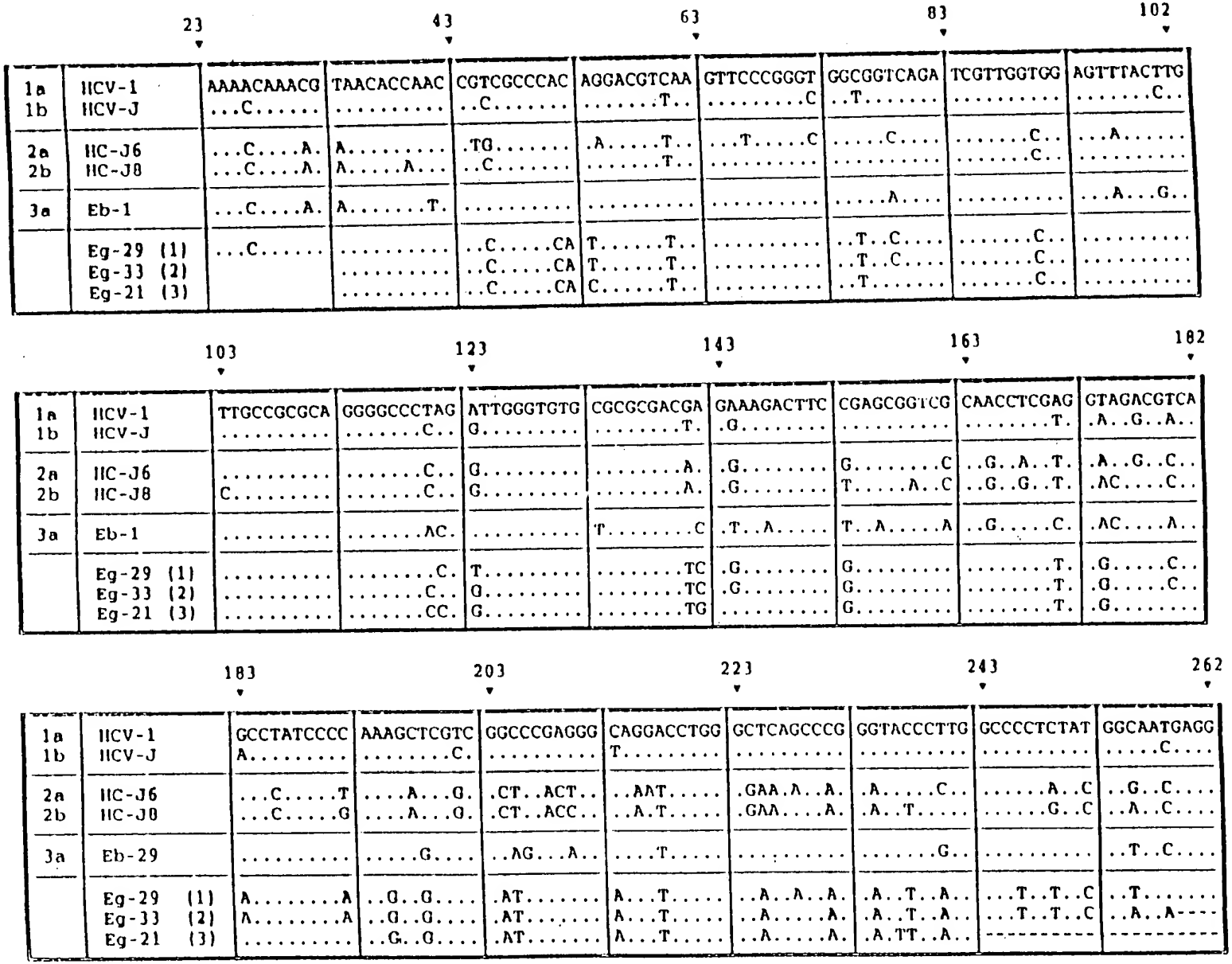

23 43 63 83 102
1a HCV-1 AAAACAAACG TAACACCAAC CGTCGCCCAC AGGACGTCAA GTTCCCGGGT GGCGGTCAGA TCGTTGGTGG AGTTTACTTG
1b HCV-J ...C..... ....C..... .....T.. .....C ..T..... ....C..
2a HC-J6 ...C....A. A..... .TG..... .A.....T.. ...T.....C .....C.... .....C.. ...A.....
2b HC-J8 ...C....A. A.....A... ..C..... .....T.. ....C.. .....
3a Eb-1 ...C....A. A.....T. ....A.... ....A...G..
Eg-29 (1) ...C..... ....C.....CA T.....T.. ....T..C.... .....C.. .....
Eg-33 (2) ....C.....CA T.....T.. ....T..C.... .....C.. .....
Eg-21 (3) ....C.....CA C.....T.. ....T..... .....C.. .....
103 123 143 163 182
1a HCV-1 TTGCCGCGCA GGGGCCCTAG ATTGGGTGTG CGCGCGACGA GAAAGACTTC CGAGCGGTCG CAACCTCGAG GTAGACGTCA
1b HCV-J ....C.. G..... .....T. .G..... ....T. .A..G..A..
2a HC-J6 ....C.. G..... .....A. .G..... G.....C ..G..A..T. .A..G..C..
2b HC-J8 C..... .....C.. G..... .....A. .G..... T.....A..C ..G..G..T. .AC....C..
3a Eb-1 ....AC. ..... T.....C .T..A..... T..A.....A ..G.....C. .AC....A..
Eg-29 (1) ....C. T..... .....TC .G..... G..... .....T. .G.....C..
Eg-33 (2) ....C.. G..... .....TC .G..... G..... .....T. .G.....C..
Eg-21 (3) ....CC. G..... .....TG ..... G..... .....T. .G.....
183 203 223 243 262
1a HCV-1 GCCTATCCCC AAAGCTCGTC GGCCCGAGGG CAGGACCTGG GCTCAGCCCG GGTACCCTTG GCCCCTCTAT GGCAATGAGG
1b HCV-J A..... .....C. ..... T..... ....C....
2a HC-J6 ...C.....T ....A...G. .CT..ACT.. ..AAT..... .GAA.A..A. .A.....C.. .....A..C ..G..C....
2b HC-J8 ...C.....G ....A...G. .CT..ACC.. ..A.T..... .GAA....A. .A..T..... .....G..C ..A..C....
3a Eb-29 ....G.... ..AG...A.. ....T..... ....G.. ....T..C....
Eg-29 (1) A.....A ..G..G.... .AT..... A...T..... ..A..A..A. .A..T..A.. ...T..T..C ..T.....
Eg-33 (2) A.....A ..G..G.... .AT..... A...T..... ..A.....A. .A..T..A.. ...T..T..C ..A..A----
Eg-21 (3) ....G..G.... .AT..... A...T..... ..A.....A. .A.TT..A.. ---------- ----------

FIGURE 15b

		5	25	45	65	85				
1a 1b	HCV-1 HCV-J	PKPQKKNKRN ....R.T...	TNRRPQDVKF	PGGGQIVGGV	YLLPRRGPRL	GVRATRKTSE	RSQPRGRRQP	IPKARRPEGR	TWAQPGYPWP	LYGNE .....
2a 2b	HC-J6 HC-J8	....R.T... ....R.T...	....	....	....	....	....	...D..ST.K ...D..ST.K	S.GK..... S.GK.....	....
3a	Eb-1	A...R.T...	.I.....	....	.V.....	..C.....	....	.....S...	S.....	.....
	Eg-29,33 Eg-21	.R..... ...	.....M.... .....T....	....	....	....G....	....	.....S... .....S...	S..... S.....F...	....---

FIGURE 16

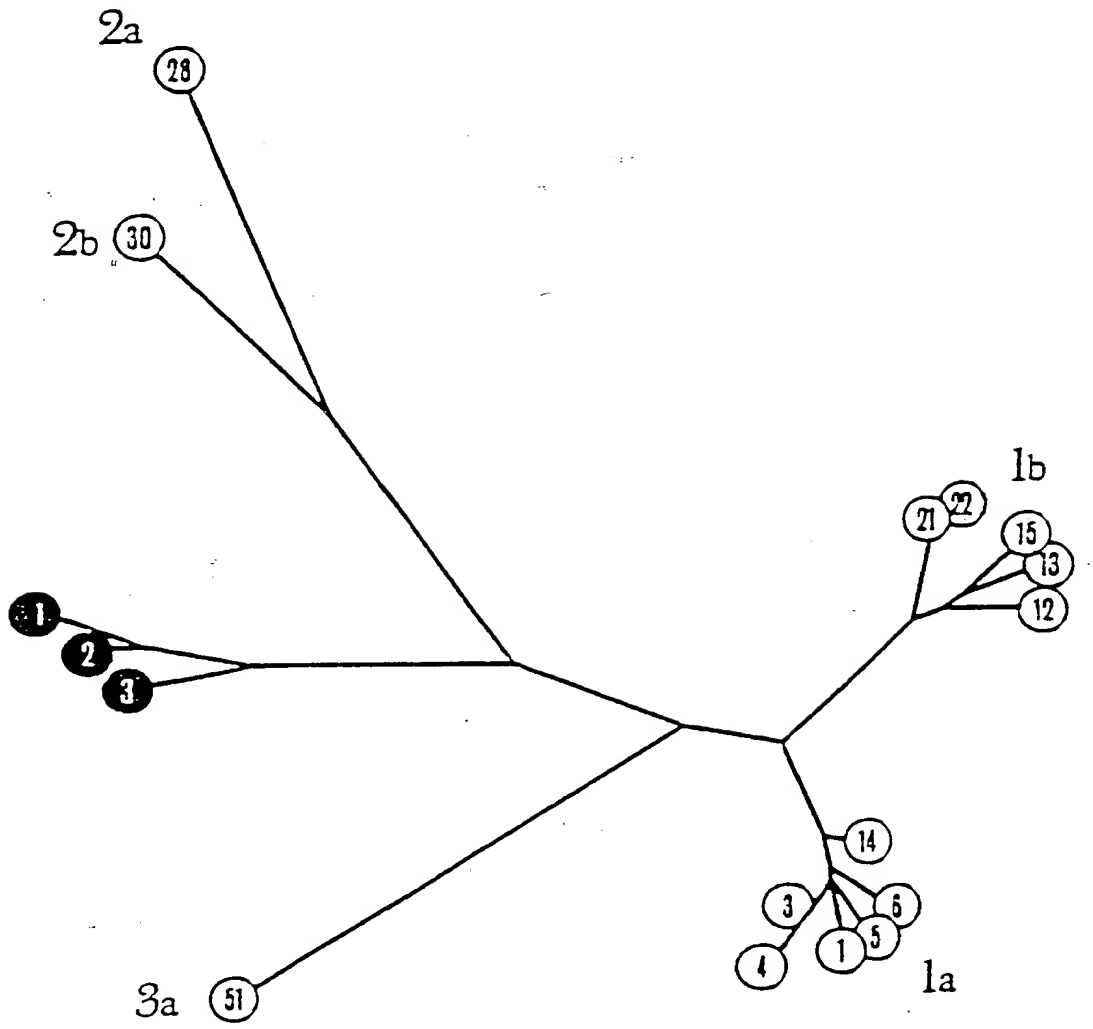
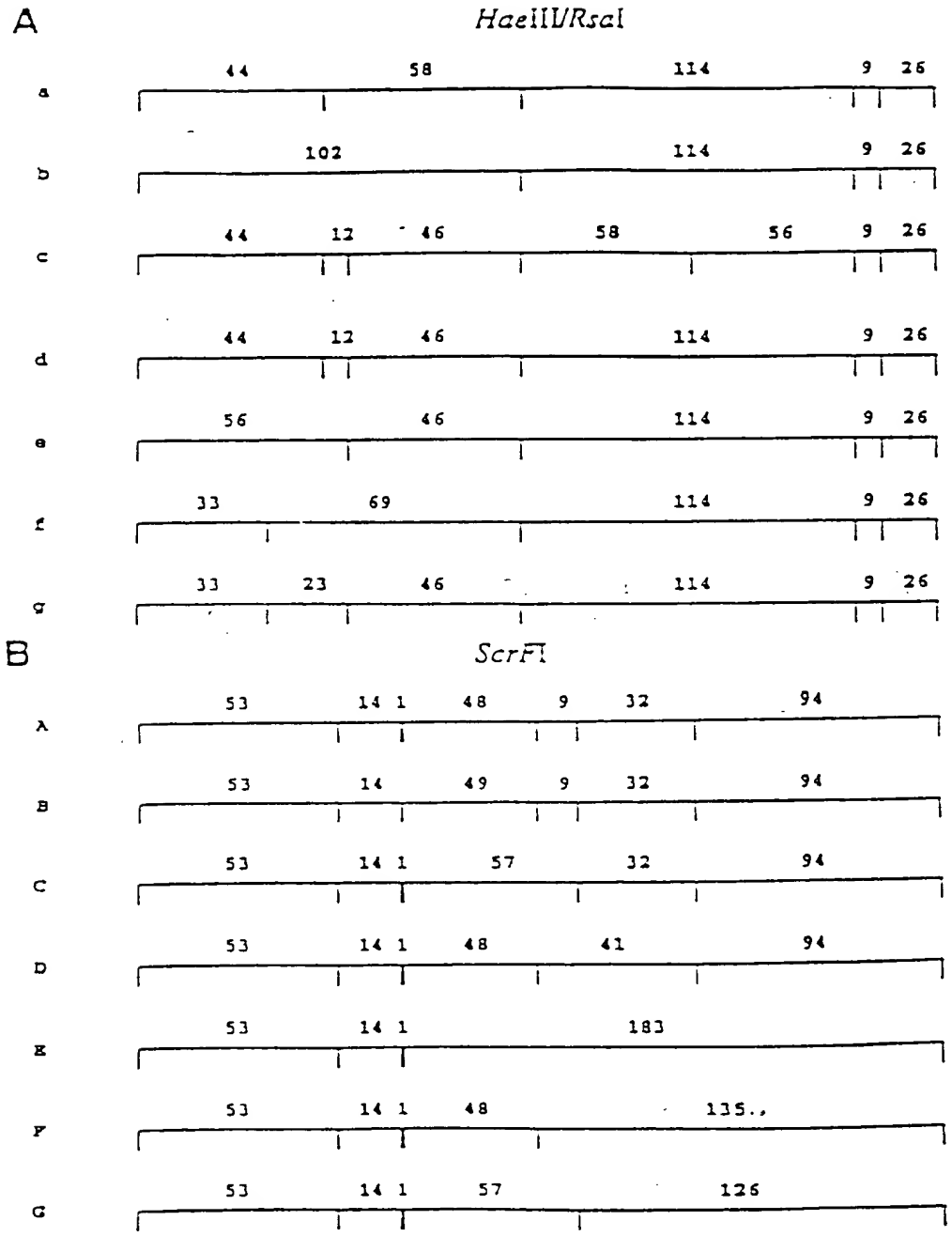


FIGURE 17


A
HaeIII/RsaI
a 44 58 114 9 26
b 102 114 9 26
c 44 12 46 58 56 9 26
d 44 12 46 114 9 26
e 56 46 114 9 26
f 33 69 114 9 26
g 33 23 46 114 9 26
B
ScrFI
A 53 14 1 48 9 32 94
B 53 14 49 9 32 94
C 53 14 1 57 32 94
D 53 14 1 48 41 94
E 53 14 1 183
F 53 14 1 48 135.,
G 53 14 1 57 126

OPERTY ORGANIZATION
l Bureau

DER THE PATENT COOPERATION TREATY (PCT)

) International Publication Number: WO 93/10239

C12N 15/51, 15/62, A61K 39/29
C12Q 1/68, C07K 13/00, 15/28
G01N 33/576

A3

(43) International Publication Date: 27 May 1993 (27.05.93)

(21) International Application Number: PCT/GB92/02143

(22) International Filing Date: 20 November 1992 (20.11.92)

(30) Priority data:
9124696.7 21 November 1991 (21.11.91) GB
9213362.8 24 June 1992 (24.06.92) GB

(71) Applicant *(for all designated States except US)*: COMMON SERVICES AGENCY [GB/GB]; Trinity Park House, South Trinity Road, Edinburgh EH5 3SE (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : SIMMONDS, Peter [GB/GB]; 1 Glengyle Terrace, Edinburgh EH3 9LL (GB). CHAN, Shui-Wan [GB/GB]; 10 Kilmaurs Road, Edinburgh (GB). YAP, Peng, Lee [GB/GB]; 5 Meadow Place, Edinburgh EH9 1JZ (GB).

(74) Agents: McCALLUM, William, Potter et al.; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).

(81) Designated States: AU, CA, FI, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

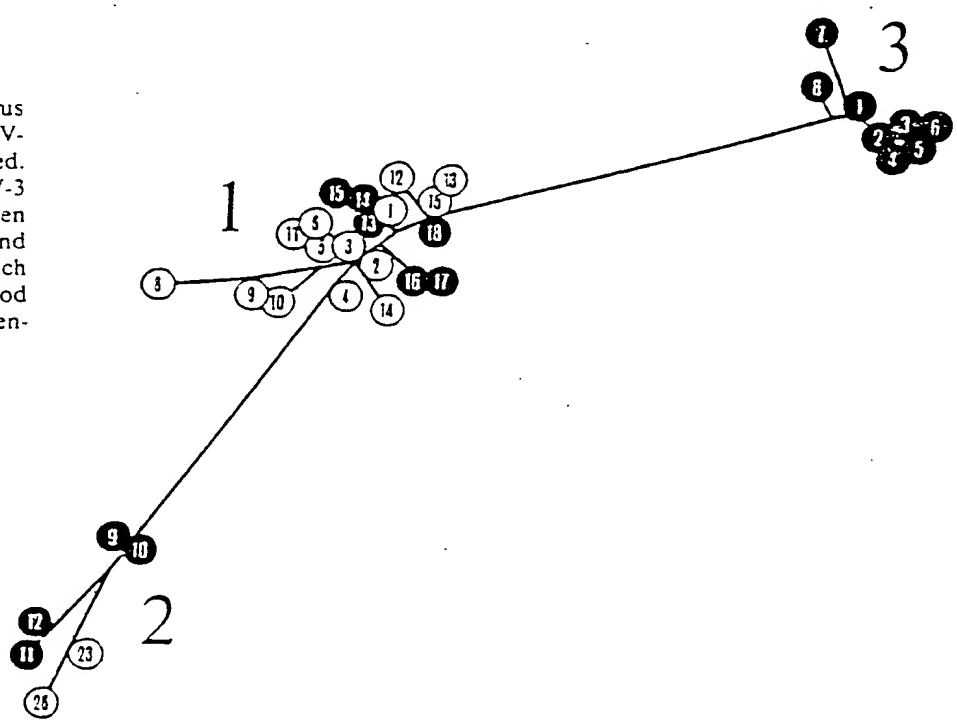
Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
22 July 1993 (22.07.93)

(54) Title: HEPATITIS-C VIRUS TESTING

(57) Abstract

New styles of hepatitis-C virus (HCV), referred to as HCV-3 and HCV-4, have been identified and sequenced. Antigenic regions of HCV-2, HCV-3 and HCV-4 polypeptides have been identified. Immunoassays for HCV and antibodies thereto are described, which allow more complete screening of blood samples for HCV, and allow HCV genotyping.



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/02143

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 12 N 15/51 C 12 N 15/62 A 61 K 39/29
C 12 Q 1/68 C 07 K 13/00 C 07 K 15/28 G 01 N 33/576

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols		
Int.Cl.5	C 07 K A 61 K	C 12 N G 01 N	C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [9]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0318216 (CHIRON CORPORATION) 31 May 1989 cited in the application see page 5, line 44 - page 7, line 17 ---	1-7,9-13,15-34
A	EP,A,0398748 (CHIRON CORPORATION) 22 November 1990 cited in the application see page 19, line 55 - page 23, line 8 ---	1-7,9-13,15-34
A	EP,A,0414475 (CHIRON CORPORATION) 27 February 1991 cited in the application see page 14, line 19 - page 15, line 1 ---	1-7,9-13,15-34
A	EP,A,0445423 (ABBOT LABORATORIES) 11 September 1991 cited in the application see page 3, line 31 - page 4, line 38 --- -/-	1-7,9-13,15-34

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12-03-1993	28 -06- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	H. HORNIG

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	J. GEN. VIROL. vol. 73, no. 5, May 1992, SOC. GEN. VIROL., COLCHESTER, UK; pages 1131 - 1141 S.-W. CHAN ET AL. 'Analysis of a new hepatitis C virus type and its phylogenetic relationship to existing variants' cited in the application see page 1139, left column, line 1 - page 1140, right column, line 6; figures 1-7	1-7,9-13,22,25,28,29
T	VIROLOGY vol. 188, no. 1, May 1992, ACADEMIC PRESS, NEW YORK, US; pages 331 - 341 H. OKAMOTO ET AL. 'Full-length sequence of a hepatitis C virus genome having poor homology to reported isolates: Comparative study of four distinct genotypes' see page 339, right column, line 16 - line 21; figure 3	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9202143 SA 67339

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 07/06/93
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0318216	31-05-89	AU-A-	2796789	14-06-89
		GB-A-	2212511	26-07-89
		JP-T-	2500880	29-03-90
		WO-A-	8904669	01-06-89
EP-A- 0398748	22-11-90	AU-A-	5812390	18-12-90
		CA-A-	2017157	18-11-90
		JP-T-	5500155	21-01-93
		WO-A-	9014436	29-11-90
EP-A- 0414475	27-02-91	AU-A-	6344990	03-04-91
		CA-A-	2064705	26-02-91
		WO-A-	9102820	07-03-91
EP-A- 0445423	11-09-91	AU-A-	6839090	27-06-91
		JP-A-	4253998	09-09-92